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VARIATION IN TICK HOST PREFERENCE AND ITS EPIDEMIOLOGICAL IMPACT

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VARIATION IN TICK HOST PREFERENCE AND ITS EPIDEMIOLOGICAL IMPACT

by

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Dedication

To my parents, who instilled in me both a love of the outdoors and of learning, and to my little sister who taught me how to work hard for the things that are important.

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Last but not least, thanks to my family. My parents, grandparents, and sister have been supportive of me my whole life, and I say with certainty that I owe any successes in life to them. I am terribly lucky to have such wonderful family, and knowing they are always on my side is a source of great comfort.

VARIATION IN TICK HOST PREFERENCE AND ITS EPIDEMIOLOGICAL IMPACT

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Tick-borne pathogens pose a significant health risk to humans and wildlife. The complex interactions between ticks and their hosts make management of tick-borne pathogens particularly challenging. Many of the most common species of ticks feed on a wide variety of hosts, but transmit pathogens that are only capable of infecting a narrow range of susceptible host species. Prior research has focused on understanding which tick hosts are capable of serving as pathogen reservoir hosts by carrying and transmitting tick-borne pathogens. However, relatively little attention has been given to studying how ticks choose their hosts. Host choice is of particular importance to the epidemiology of tick-borne pathogens when not all hosts are pathogen reservoirs.

My dissertation research investigates the nature of host choice and its impact on disease prevalence in two tick species with similar life histories and host ranges: the lone star tick (*Amblyomma americanum*) and the American dog tick (*Dermacentor variabilis*). I conducted an experiment to demonstrate that lone star ticks can respond to host scent. Certain host scents, including those from some individual opossums and raccoons, are attractive to ticks. Proximity to scent also influences tick movement. I also looked for evidence that American dog tick populations are genetically structured by host species

identity, and found that certain tick genotypes correlate with host species. This suggests that these ticks may have heritable host preferences that influence their feeding behaviors. Finally, I used a mathematical model to predict disease transmission probability and lone star tick preference for reservoir hosts. I considered hypothetical wildlife communities with different reservoir host relative abundances, and found that changes in relative abundance influence both disease transmission probability and tick host preference estimates. The model also suggests that lone star ticks must parasitize reservoir hosts more frequently when those hosts are less common. These results highlight the importance of host choice and host community composition as determinants of tick-borne disease prevalence.

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Chapter 1: Introduction

Tick-borne disease has a great impact on human health. Approximately 30,000 people contract Lyme disease each year (Kuehn, 2013), and spotted fever rickettsiosis, babesiosis, anaplasmosis, and ehrlichiosis infected a total of almost 9,000 people in 2012 (Adams et al., 2014). These diseases are zoonotic – caused by pathogens typically harbored in wildlife populations. The impact of tick-borne disease on wildlife population health is largely unknown, but the role of wildlife in the epidemiology of tick-borne pathogens is unquestionable: circulation of tick-borne diseases in wildlife populations allows spillover of these diseases into human populations (Daszak, 2000). Understanding tick-borne pathogen transmission in wildlife populations is critical to understanding the greater transmission patterns that put people at risk.

TICK-HOST INTERACTIONS MEDIATE PATHOGEN SPREAD

Pathogens are spread between ticks and wildlife hosts when ticks feed on these animals (Paddock & Childs, 2003). Ticks and pathogens both have their own vertebrate host ranges: ticks have a set of vertebrate species that are acceptable as hosts, and pathogens have a set of vertebrate species they can infect (Childs & Paddock, 2003; Paddock & Childs, 2003; Sonenshine, 1991). If a vertebrate species can become infected with a tick-borne pathogen and infect ticks that may subsequently feed on it, that species is said to be a pathogen reservoir. Pathogen reservoirs are critical components for the maintenance of endemic tick-borne disease infection in wildlife communities (Haydon, Cleaveland, Taylor, & Laurenson, 2002).

Not all animals can act as reservoirs for tick-borne pathogens – non-reservoir hosts are transmission dead-ends, and pathogens can be regarded as specialists with respect to their host use (Paddock & Childs, 2003; Parola & Raoult, 2001) On the other

hand, some ticks are prolific feeders with wide host ranges. When ticks have host ranges are larger than their pathogens host ranges, transmission of tick-borne pathogens in wildlife communities is highly dependent on the wildlife community composition (LoGiudice, Ostfeld, Schmidt, & Keesing, 2003). The disease dilution hypothesis hypothesizes that when wildlife community diversity is increases, the relative abundance of reservoir species decreases. Ticks are then less likely to encounter a reservoir animal and disease prevalence is expected to decrease in tick and reservoir animal populations (LoGiudice, Ostfeld, Schmidt, & Keesing, 2003).

The dilution hypothesis presumes that ticks are true generalists and that all of their hosts hold equal value (McCoy, Léger, & Dietrich, 2013). This allows disease prevalence in tick and reservoir populations to be a direct consequence of community diversity. What if not all tick hosts have the same value, and ticks prefer some hosts to others? Extrapolating from the disease dilution model, if ticks prefer to feed on pathogen reservoirs, increasing community diversity may have little effect on disease prevalence. Conversely, if ticks prefer non-reservoir hosts, disease dilution could be accelerated.

The notion of host preference in certain tick species is based on some general observations about foraging patterns and tick burdens. Even within taxa that are regarded as generalists, tick burdens vary by host species and even by individual host. For example, lone star ticks (*Amblyomma americanum*) are found more commonly on white-tailed deer, raccoons and coyote than on other wildlife species (reviewed both in Bishopp & Trembley, 1945 and Childs & Paddock, 2003). The vector of Lyme disease (*Ixodes scapularis*), despite its assumed indifference to hosts, is noted to feed more commonly on reptiles in field studies, and to prefer mice and reptiles to chicken in laboratory studies (Apperson, Levine, Evans, Braswell, & Heller, 1993; James & Oliver, 1990). The

mechanism that underlies these host choices remains unknown, and the interaction between community structure and these choices has not been well-studied.

Conceptual Model for Tick-Host Interactions

Many of the common disease-carrying ticks are three-host ticks. These ticks have three feeding life stages (larva, nymph, and adult; Figure 1.1), and feed on a different host individual during each life stage. Questing strategies vary by tick species, and involve either ambushing or hunting host animals. Ambush hunters ascend vegetation and wait for passing hosts. Hunters may do the same, but will also move quickly through the leaf litter or vegetation to reach a nearby host rather than wait for the host to pass. Both host finding strategies are believed to rely on general cues: movement, CO_2 output from host respiration, and passing shadows indicate to ticks the presence of a host (Sonenshine, 1991).

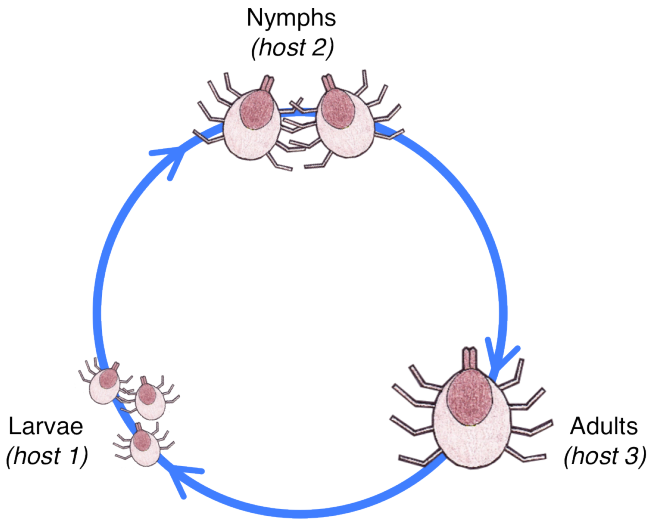

Figure 1.1: Three-host life cycle.
Ticks feed on host 1 as larvae, on host 2 as nymphs, and on host 3 as adults. Disease transmission may occur during any of these feeding events. Ticks spend the time between feeding events off-host, in the leaf litter digesting their blood meals.

Very little is known about the on-host behavior of ticks; that is, what ticks do between finding a host and attaching to feed. Ticks tend to aggregate in certain areas of the host body (e.g., ears), and this is largely due to the release of aggregation pheromones by ticks already feeding at those sites (Brunner & Ostfeld, 2008; Diehl, Guerin, Vlimant, & Steullet, 1991; Rosà, Pugliese, Norman, & Hudson, 2003; Sonenshine, 1991). Ticks appear to express negative gravitaxis on vegetation and perhaps also on hosts (Lees, 1948; Semtner & Hair, 1973). Still, it is a long journey from the leg to ear on a deer, and ticks may be receptive to other cues about host quality that are informative in deciding whether to stay on the host and feed or drop off in search of a better meal.

My dissertation encompasses three projects that aim to demonstrate that tick host choice exists and has epidemiological consequences. I consider three questions that correspond with the subsequent chapters.

(A) Can ticks discern host identity using scent as an informative cue? Host scent may convey information about species identity and other markers of host quality like immune status. If ticks are responsive to host scent, it may be a cue used in foraging decision making.

(B) Does tick population genetic structure show evidence of persistent host preference? Many species of ticks mate on their hosts, limiting the set of available mates to only those ticks on the same host animal. If ticks prefer certain host species, and particularly if host preference is variable within tick populations, those host preferences may lead to reproductive isolation of ticks by host species.

(C) Can host preference be detected using epidemiological data? A wealth of disease prevalence data exist in the literature, and these data are used to parameterize a mathematical model describing tick-borne disease

transmission in a wildlife community and make inferences about expected feeding behaviors given observed patterns of prevalence.

STUDY SYSTEM: THREE-HOST TICKS OF EAST TEXAS

I address these questions using two species important disease vectors: the lone star tick (*Amblyomma americanum*) and the American dog tick (*Dermacentor variabilis*). Both species parasitize commonly found wildlife species as well as humans, leading to infection risk for humans in areas where tick populations and endemic disease prevalence are high. These ticks also both mate on their host animals. Mating on hosts may reinforce heritable host preferences by limiting available mates to those sharing the same preference.

The Lone Star Tick, *Amblyomma americanum*

The lone star tick is ubiquitous in the eastern and southeastern United States. Lone star ticks are regarded as prolific and aggressive feeders, and have been documented on over 30 different vertebrate species including mammals, reptiles, and birds (Bishopp & Trembley, 1945; Sonenshine, 1991). Of the many pathogens carried by lone star ticks, *Ehrlichia chaffeensis* has emerged in recent decades as a significant threat to human health (Paddock & Childs, 2003). White-tailed are the vertebrate reservoir for *E. chaffeensis* (Ewing et al., 1995; Lockhart, Davidson, Stallknecht, Dawson, & Howerth, 1997). Other animals are known to be naturally exposed to *E. chaffeensis*, but their role in transmission of this pathogen has not been confirmed (Comer, Nicholson, Paddock, Sumner, & Childs, 2000; Davidson, Lockhart, Stallknecht, & Howerth, 1999; Yabsley et al., 2008).

There is spatial heterogeneity in the prevalence of *E. chaffeensis* within the range of its vector and reservoir. White-tailed deer populations often have high *E. chaffeensis* infection prevalence, ranging up to 26% (though some populations are seemingly uninfected) (Little, Stallknecht, Lockhart, Dawson, & Davidson, 1998; Lockhart et al., 1997; Yabsley et al., 2004). Lone star ticks, on the other hand, have typically lower infection prevalence ranging up to 10% (Mixson et al., 2006; Varela, Moore, & Little, 2004; Whitlock, Fang, Durden, & Oliver, 2000). My dissertation research seeks in part to determine if lone star ticks feed preferentially on certain hosts within their host range, and to determine if preferential feeding can explain the observed patterns of disease prevalence in tick and wildlife populations.

The American Dog Tick, *Dermacentor variabilis*

American dog ticks are vectors of *Rickettsia rickettsii* (Azad & Beard, 1998). Unlike *E. chaffeensis*, the reservoir animals for *R. rickettsii* have not been definitively established. Likely candidates which are also hosts of American dog ticks include domestic dogs and hispid cotton rats, opossums, and cottontail rabbits (Gage, Burgdorfer, & Hopla, 1990; McDade & Newhouse, 1986; Norment & Burgdorfer, 1984; Shirai, Bozeman, Humphries, Elisberg, & Faber, 1967). This pathogen can be transmitted vertically. However, after several generations the continued infection leads to lowered reproductive output and infection-induced mortality in ticks (Niebylski & Schwan, 1999). Therefore some transmission from reservoir host animals is required to maintain endemic *R. rickettsii* infection in tick populations (McDade & Newhouse, 1986; D. Walker & Fishbein, 1991; D. H. Walker, Paddock, & Dumler, 2008).

The American dog tick has been documented on approximately 52 different host species, far more than the number of species considered potential *R. rickettsii* reservoirs

(Bishopp & Trembley, 1945). My dissertation research investigates whether these ticks are reproductively isolated by their host choices. Such reproductive isolation would indicate that ticks have heritable host preferences that influence feeding behaviors and give rise to genetically subdivided populations.

EPIDEMIOLOGICAL IMPORTANCE OF HOST PREFERENCE

The research described in this dissertation helps establish that some ticks make non-opportunistic, non-generalist foraging decisions with potential epidemiological consequences. Ticks experience variation in available hosts that may influence foraging decisions, and those decisions may have epidemiological consequences. If ticks are not opportunistically feeding, then the opportunity for disease dilution in more diverse wildlife communities is mitigated. Furthermore, the discovery that ticks feed non-opportunistically may lead to novel methods of tick population control. Animal species that are the most attractive to ticks may bear a disproportionate tick burden and contribute heavily to the maintenance of tick populations. Once the mechanisms for host choice have been established, they can be integrated into the framework for describing tick-borne disease spread in wildlife communities. Targeted treatment of preferred host animals may be a means by which tick populations can be controlled and disease risk, including the risk of spillover infections in human populations, can be reduced.

Chapter 2: Response of nymphal lone star ticks to animal scent

ABSTRACT

Ticks utilize a variety of strategies for detecting hosts in their environment. They detect host movement by sensing vibrations in vegetation, respiration through CO_2 gradients, and passing animals through visual cues. These general host detection mechanisms do not explain the observed differences in tick burdens between different host species. Anecdotal evidence suggests that the lone star tick, *Amblyomma americanum*, exercises preference for certain host species, and previous studies have suggested host scent as a mechanism by which host-specific feeding decisions could be made. I conducted two experiments to assess the response of nymphal lone star ticks to raccoon, opossum, bobcat, and white-tailed deer scent. I found that ticks respond positively to raccoon and opossum scent, but not to bobcat and white-tailed deer scent, that some opossum scents are more favorable than others, and that proximity to host scent is more important than the identity of the host scent. These results suggest that preferences may help to explain variable tick burdens, and tick use of host-specific cues should be included in the framework for describing tick foraging behavior.

INTRODUCTION

Finding a host is imperative for tick survival; host animals provide both nourishment and reproductive opportunities. Ticks use a number of strategies for finding hosts: detection of CO_2 gradients, detection of shadows in tick species with eyes, and detection of movement as hosts move through vegetation (Sonenshine, 1991). These cues are largely non-specific with regard to host species identity. With the exception of body size influences on CO_2 output and movement behaviors, all host species are equally

detectable by these cues. However, some host species are more highly parasitized than others, raising the question of whether ticks are selective in their choice of hosts (Brunner & Ostfeld, 2008; Childs & Paddock, 2003; Schulze, Jordan, & Hung, 2001; Schulze & Jordan, 2003).

The lone star tick, *Amblyomma americanum*, is a generalist tick that has been documented on over 30 vertebrate species. However, anecdotal reports suggest that lone star ticks are somewhat selective in their choice of host animals. White-tailed deer are regarded as the primary host of lone star ticks, and dogs, raccoons and coyotes have been suggested as additional preferred hosts. It has also been hypothesized that preferences change with life stage, and that smaller instars (larvae and nymph) prefer smaller host animals (Childs & Paddock, 2003; T. M. Kollars, Oliver, Durden, & Kollars, 2000).

Preference has not been well-defined in the context of lone star tick feeding choices, and the assertions of preference are founded primarily on tick burden observations made without knowledge of the underlying wildlife community composition. Host availability and wildlife community structure may dramatically impact the observed patterns of parasitization. Therefore, additional work is necessary to understand if lone star tick preferences truly exist or are an artifact of incomplete sampling.

Preference of lone star ticks for different host species can be directly studied by looking at the responses of these ticks to host-specific cues. If lone star ticks in fact choose certain host species to parasitize, they must use some cue to discern the identity of the host. Scent has been proposed as a host-specific cue, and several studies have demonstrated that ticks of several different species will walk toward the scent of host animals. Adult lone star ticks (*Amblyomma americanum*), blacklegged ticks (*Ixodes scapularis*), and American dog ticks (*Dermacentor variabilis*) all show attraction to dog

and deer scents (Carroll, 2002). Nymphal sheep ticks (*Ixodes ricinus*) also respond positively to the scent of dogs (Crooks & Randolph, 2006).

Given that lone star ticks have a diversity of possible host animals, studies into their scent responses can extend beyond white-tailed deer and dogs. The scent response study conducted by Carroll (2002) utilized scent from a single deer and a single dog. I extend this work to include scent from multiple different animal species, and multiple individuals of some species where possible.

The life stage considered is also important. I focus on only the nymphal life stage because ticks that acquire infection during the nymphal life stage or before have more opportunities to transmit disease to subsequent host animals than if infection is acquired in the terminal (adult) life stage. White-tailed deer are the primary reservoir *E. chaffeensis*, a pathogen spread by lone star ticks (Lockhart et al., 1997), so the response of nymphal lone star ticks to scent from deer and other host species has epidemiological relevance.

The previous research on tick response to animal scent has relied on the use of relatively simple statistical analysis of binomial outcome trials in single-scent experiments where animal scents were paired with unscented items. In this case, the ticks do not have a choice between multiple scents, but instead may respond or not respond to the only scent available. Alternatively, "choice" experiments can pair different scents together. Choice experiments are less reflective of the natural foraging conditions observed by ticks, but have the power to show whether ticks respond differently to scents from different animals (Roa, 1992). I used a combination of single-scent and choice experiments to evaluate the response of nymphal lone star ticks to raccoon, white-tailed deer, opossum, and bobcat scents. All four of these animals are common wildlife species and documented hosts of lone star ticks (Bishopp & Trembley, 1945; Childs & Paddock,

2003). Using scent from more than two animals to conduct experiments requires the application of more sophisticated statistical analysis. I adapted Bradley Terry paired comparison models to estimate the ability of individual animal scents to attract nymphal ticks in these experiments (Bradley & Terry, 1952).

I conducted scent response experiments to assess the hypotheses (1) lone star tick nymphs will respond positively to scents from all of these animals, (2) proximity to scent will influence their choices, and (3) they will prefer white-tailed deer scent. I used scent samples from individuals representative of these species and assayed tick behavior in a custom-built scent response arena.

METHODS

Animal Scent Collection

I obtained scents directly from 3 raccoons, 3 opossums, 1 bobcat, and 1 white-tailed deer. Scents were collected from animals on sterile 5.0cm x 5.0cm gauze sponges. Gauze sponges were rubbed on animal abdomens for 10s to standardize the amount of scent in the absence of well-established means for quantifying scent collection from animals. Scented gauze sponges were placed in Ziploc bags and stored on dry ice for transport back to the lab, then stored at -80^{O}C until use (Crooks & Randolph, 2006). This is consistent with other scent studies that have used gauze for scent collection (Crooks & Randolph 2006) and cold temperatures to prevent the diffusion of volatile compounds that may be components of animal scent (Carroll, 2002). Latex gloves were used in the handling of gauze samples and sample bags to prevent contamination with human scent.

Raccoons, opossums and bobcats were live-trapped at Gus Engeling Wildlife Management Area in Tennessee Colony TX in October 2011. Ten wire mesh live traps

(Tomahawk Live Trap, Hazelhurst, WI) were baited with fish-flavored cat food and placed a transect at 100m intervals in a riparian corridor. Traps were set out at dusk and checked at dawn for a total of 30 trap-nights. Trapped animals were anesthetized with a combination of ketamine and dexmedetomidine in accordance with established procedures and dosages (Baldwin, Winstead, Hayden-Wing, Dreeger, & Dzialak, 2008; Fowler, 2008). Dosages are listed in Appendix A. All handling of live animals was done under the guidance and approval of The University of Texas at Austin Institutional Animal Care and Use Committee (IACUC) under protocol #AUP-2011-00057.

Gus Engeling Wildlife Management Area conducts public hunts, and all harvested deer must be checked by area officials as part of the deer herd management program. White-tailed deer scent was obtained from a hunter-harvested deer in November 2011 using the gauze-collection protocol described above.

Nymphal Lone Star Tick Acquisition & Storage

Laboratory-reared nymphal lone star ticks were acquired from the Oklahoma State University Tick Rearing Facility. All nymphs were from the same clutch of eggs (siblings) had been fed on rabbit as larvae on 14 July 2011 and molted to nymphs on 20 August 2011. Nymphs were received on 28 October 2011 and were stored according to the Tick Rearing Facility recommendations until use in the scent response assay: 23^{O}C, 15h light:9h dark at 95% humidity. All trials were conducted within 6 weeks of tick receipt, making the maximum age of nymphs used in the experiment 3.5 months post-molt. Nymphal ticks may spend up to 10 months questing for hosts after molting, so age of the ticks participating in the experiment was within the expected range for this behavior (Haile & Mount, 1987).

Scent Response Assay

Arena Design

I designed and constructed two scent response assay arenas in which to conduct the experiment. Each arena was fabricated out of glass and measured 57cm long x 6cm wide x 6cm deep. A piece of glass with slightly larger dimensions was placed on top to create a fully enclosed space. Ticks were prevented from escaping by a moat built into the bottom of the arena. A raised island made of glass was affixed to the bottom of the arena so that a 0.5cm space separated the island from the walls of the arena. The moat was filled with water to prevent ticks from reaching the vertical walls of the arena, climbing up, and escaping under the lid of the arena. The arena was placed on a fiberglass light box for easier detection and tracking of movement. A video camera was suspended over the arena to capture ticks in motion (Figure 2.1).

Experimental Design

I placed ticks and scent samples in the scent response arena and recorded which gauze sample was visited first by each tick. For each trial, I placed three ticks in different positions inside the arena (left, middle, and right) to see if proximity to the scent sample influenced tick movement. There were approximately 12cm between each of the ticks, and between the ticks on the ends and the gauze samples. Each tick participated in only one trial, and individual variation in scent response was not assessed. The location of the scents (left or right side of the arena) were switched between trials to control for any positional preferences ticks may have independent of scent stimuli. Ticks were allowed to move in the arena for up to 30 minutes before removal. Ticks that did not make a choice in that time period were excluded from analysis. The scent arenas were cleaned with 10% bleach and rinsed with DI water between each trial to prevent scent carry-over between

trials. Trials were conducted in a closed, dark room with the light box as the only source of light. All trials were conducted between 16 November 2011 and 12 December 2011.

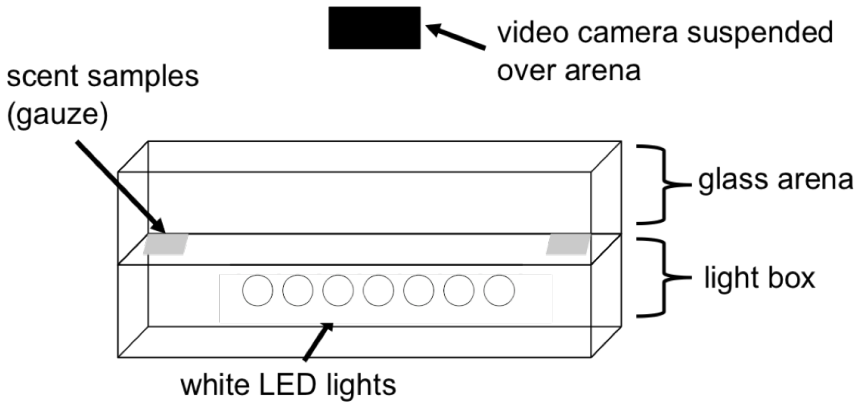

Figure 2.1: Scent response arena schematic.

Single-Scent Trials Ticks were presented with scented and unscented gauze to see if they could detect animal scents (Figure 2.2). Because the animal-scented gauze samples had been handled by latex-gloved hands, the unscented gauze samples also handled with latex gloves so that any latex scent acquired by scented samples was also shared by unscented samples. The unscented gauze samples were also placed in the -80^{O}C freezer prior to use. All gauze samples were brought to room temperature before being placed in the scent arena. Appendix B lists the individual scents used in single-scent trials.

Choice Trials Ticks were presented with pairs of scents from individual animals of different species. All scent samples were brought to room temperature before use and handled with latex gloves. Appendix B lists the individual scent combinations used in choice trials.

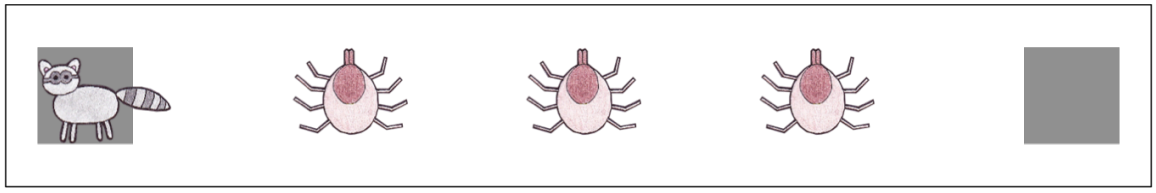

Figure 2.2: Single-Scent experimental set-up.
Ticks are equally spaced in the middle of the arena, and scents are placed at either end in a block design to control for scent position.

Analysis

The video camera was connected to a laptop computer, and video was captured using Debut v1.52 (NCH Software), and subsequently analyzed using ImageJ v1.44 (Abramoff, Magalhaes, & Ram, 2004). Choice was determined as the tick crossing leading edge threshold of the gauze sample (Figure 2.3). Ticks were not required to climb on the gauze or stop moving near the gauze.

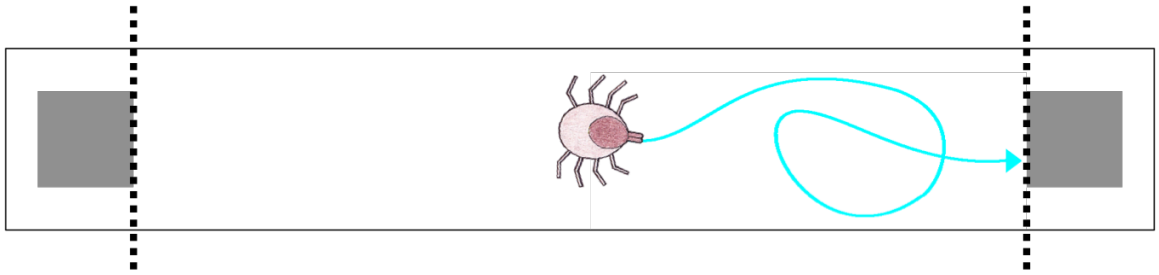

Figure 2.3: First choice tick responses.
Black dashed lines represent indicate the leading edge threshold of the gauze sample.

Single-scent and choice experiments were analyzed separately to address two independent questions: do ticks respond to these host scents, and do ticks have preference for certain scents? Because tick responses to scent may vary both by species and by

individual, and because each species was represented by only 1-3 individuals, it is difficult to disentangle the attractive effect of species from the attractive effect of individual (Singer & Lee, 2000). Consequently, subsequent analyses take the more conservative approach of determining the attractive influence of individual animals rather than species groups.

Bradley-Terry paired comparison models were used to analyze the results from both single-scent and choice experiments (BradleyTerry2 package; R version 3.0.2) (R Core Team, 2013; Turner & Firth, 2012). Bradley Terry paired comparison models are generalized linear models that estimate the probability of scent *i* attracting ticks over the set of other scents *j* is modeled as α_i/α_i using maximum likelihood methods (Bradley & Terry, 1952; Turner & Firth, 2012). Each trial has a binomial outcome (choice for scent *i* or choice for scent *j*), and ticks that do not make a choice are excluded from the analysis.

Bradley Terry paired comparison models are not typically used in analysis of preference studies because they require an assumption of independence. Food preference studies typically fail to meet this assumption because subjects consume some of the selected food resource and therefore change its value relative to the alternatives by simultaneously reducing the amount of food available and satiating hunger (Lockwood III, 1998; Manly, 1993; Roa, 1992). However, because only the first choice is measured and because the value of the scent stimuli is not altered when a tick selects it, the Bradley Terry paired comparison model is suitable for this application.

RESULTS

Single-Scent Trials

Ticks were presented with scent from raccoon 1, raccoon 2, opossum 1, bobcat, and deer paired with unscented gauze. Ticks chose the scented gauze sample (regardless of the individual scent used) more often than the unscented gauze sample when they were positioned both near and far from the scented sample, though ticks in the middle position chose scented and unscented gauze with approximately equal frequency (Figure 2.4). I conducted a χ^2 contingency test to show no association between first choices (scent or no scent) and tick starting position ($\chi^2=2.57$, $df=2$, $p=0.28$). This suggests no impact of tick starting position on the choice made in the single-scent trials.

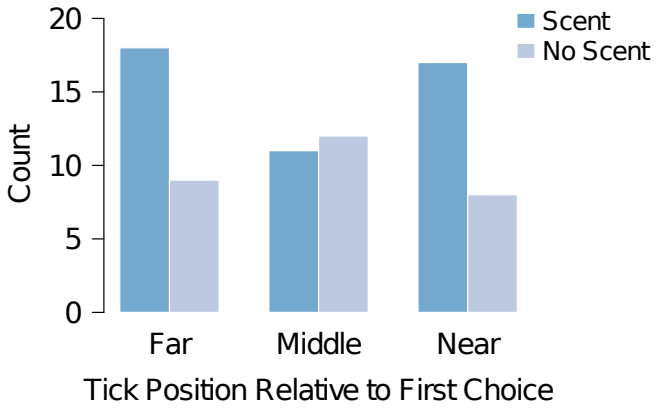

Figure 2.4: Positional responses to animal scent, single-scent trials.
Tick positions relative to the scent stimulus were recorded as either far, middle, or near. The number of ticks first choosing the scented sample is indicated by dark blue bars, while the number choosing the unscented stimulus is indicated by the light blue bars. There is no statistically significant association between tick position relative to first choice and the first gauze sample chosen ($\chi^2=2.57$, $df=2$, $p=0.28$)

The ability of individual scents to attract ticks was estimated using a Bradley Terry paired comparison model with individual animal as the main explanatory variable. Tick starting position was not included as a main effect because of low sample size. I excluded starting position as a main effect because the χ^2 contingency suggest no influence of starting position on the results. Data from all tick starting positions were grouped together for analysis. The Bradley Terry model estimate of the attraction coefficients (α_i) shows ticks have a significant positive response to scents from raccoon 1 and opossum 1 (Figure 2.5). There was no positive response to scent from opossum 2, or to the bobcat or deer scent.

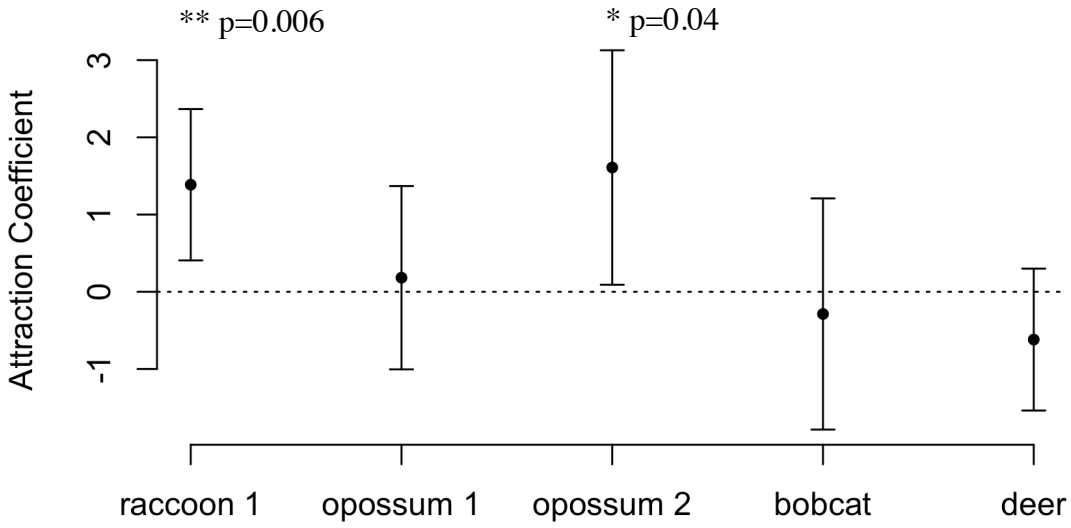

Figure 2.5: Single-Scent attraction coefficients.
The estimated attraction coefficient for each individual scent is shown, along with 95% confidence intervals. Raccoon 1 and opossum 2 are significantly attractive to ticks (p=0.006 and p=0.04, respectively). The dashed line represents an attraction coefficient of 0; estimates above that line indicate a scent is attractive while values below that line indicate a scent is unattractive.

Choice Trials

Choice trials focused on detecting preferences of nymphal ticks for different host individuals. Raccoon 1, raccoon 2, and raccoon 3 were each paired against opossum 3 in choice trials. There were not enough scent samples remaining from the bobcat or deer to conduct choice trials with sufficient power for analysis. Ticks positioned on the left side of the arena are more likely to choose the scent also positioned on the left side of the arena, regardless of the identity of the scent (Figure 2.6). Similarly, ticks positioned on the right of the arena are more likely to choose the scent on the right. This suggests the tick starting positions relative to the gauze sample first visited shows that when presented with two scent choices, ticks more frequently visit the closest scent. Ticks placed in the middle of the arena more frequently visited the left scent sample, regardless of the identity of the scent. Given the blocked design, each individual scent was placed on the left and on the right of the arena an equal number of times. The attraction of ticks positioned in the middle to the left side of the arena therefore cannot be explained by the identity of the scent.

Tick starting position has a significant effect on the scent sample chosen ($\chi^2=7.94$, $df=2$, $p=0.019$). As with the single-scent trials, there was insufficient power to analyze tick starting position as a main effect due to low sample size. Given the apparent importance of tick starting position on the outcome of the trial, and in the absence of sufficient sample size for proper statistical inference, attractive ability of individual scents was not estimated for the choice trials.

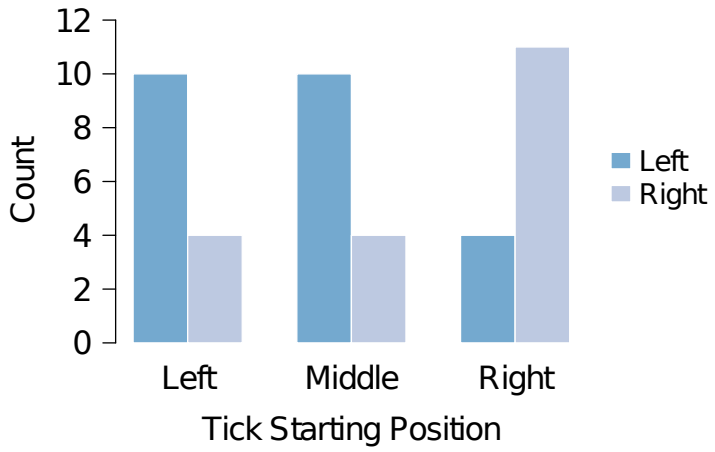

Figure 2.6: Positional responses to animal scent, choice trials.
Ticks were positioned in the left, middle, or right portion of the scent response arena, and scents were positioned on the left and right extremes of the arena. The dark blue bars indicate the number of ticks that went to the left-hand scent, while the light blue bars indicate the number of ticks that went to the right-hand scent. Tick starting position is significantly associated with first choice scent ($\chi^2=7.94$, $df=2$, $p=0.019$).

DISCUSSION

Nymphal lone star ticks are responsive to scent cues from certain host individuals, consistent with previous observations that adult lone star ticks can detect host scent as well. One raccoon and one opossum both attracted a significant number of ticks in the no-host trials. In contrast to results with adult lone star ticks (Carroll, 2002), nymphal lone star ticks were not significantly attracted to white-tailed deer. This difference may be due to the sampling methodology I employed. Scent collected from the abdomen may have different chemical composition or lower potency than the glandular scent used by Carroll in the 2002 study. The compounds secreted by host animals that ticks find attractive are not known, making it impossible to standardize the concentration of scent stimuli

presented from different species or from different individuals, so variation in the amount of attractant used may also explain this discrepancy.

There was variation in the attractiveness of opossums in the single-scent experiment. Opossum 2 was significantly attractive to ticks, but opossum 1 was not. Parasite burdens are often variable across individual animals, and host scent may very well be one source of this variation. Scent may reveal information relevant to the quality of the animal as host, including information about health and immune status. *Ixodes hexagonus* ticks appear to discern the health condition of hedgehogs using fecal scent and parasitize unhealthy individuals more frequently (Bunnel, Hanisch, Hardege, & Breithaupt, 2011). Other blood feeding arthropods, including mosquitoes, sandflies, and triatomines, detect host scent and may respond to variation in the chemical compounds secreted by different host individuals (McBride et al., 2014; Takken & Knols, 2010).

My results also suggest that proximity to scent may be more important than the identity of that scent in attracting nymphal ticks. Scent may not detectable from great distance, or it may not be a useful cue until ticks are very near their host animals. Rarely in nature would ticks be in a position to receive scent cues from multiple host animals, so cues such as movement and CO_2 emission play a significant role in host-finding. Consistent with the observations from the single-scent experiment that individuals vary in the attractiveness of their scents, the role of proximity in attracting ticks suggests that scent is a means by which ticks evaluate the quality of a host once they are already on it. Scent may be a cue used by ticks to help decide whether or not to feed or find a different host.

The existing paradigm for host finding in ticks is that ticks find hosts by non-specific cues and feed on hosts as they are encountered. My results, along with other studies on the role of olfaction in host finding, suggest on-host behavior should be added

to that paradigm. On-host behavior, specifically differential response to host scent, may be an expression of host preference or evaluation of host quality. The suggestion that variation in host quality may be responsible for tick aggregation is not new, but no compelling mechanism has been put forward to explain these differences (Brunner & Ostfeld, 2008; Childs & Paddock, 2003; T. M. Kollars et al., 2000). The on-host behavior of ticks has not been well-studied, but may offer insight into these differences. On-host tick behavior and differences in tick burdens have strong epidemiological consequences. Should host scent be influential in driving these differences in tick burden, it could be exploited as a means of tick control.

Appendix A: Sedative & Anesthetic Dosages

Animal	Avereage dexmedetomidine dose, mg/kg (average)	Average ketamine dose, mg/kg (average)
Raccoon	0.024-0.037 (0.028)	1.78-2.89 (1.97)
Opossum	0.025-0.06 (0.04)	1.6-2.0 (1.79)
Bobcat	0.03	10

Table A.1: Anesthetic and sedative dosages for scent preference study animals.

Appendix B: Experimental trials

Scent Samples		N	Scented Gauze Chosen	Unscented Gauze Chosen	No Choice Made
raccoon 1	none	30	20	5	5
opossum 1	none	12	6	5	1
opossum 2	none	12	10	2	0
deer	none	30	7	13	10
bobcat	none	24	3	4	17

Table B.1: Scents used in single-scent trials

Scent 1	Scent 2	N	Scent 1 Chosen	Scent 2 Chosen	No Choice Made
raccoon 1	opossum 1	12	7	4	1
raccoon 2	opossum 2	12	4	3	5
raccoon 3	deer	18	9	7	2
opossum 3	deer	12	4	5	3

Table B.2: Scents used in choice trials

Chapter 3: Genetic evidence of specialization and differential host use in American dog ticks

ABSTRACT

Ticks transmit disease in wildlife communities when they feed on host animals. Many ticks are presumed to be generalists, feeding entirely opportunistically on all species within their host range. Tick-borne pathogens, however, are typically specialists with few competent vector and reservoir animals. Consequently, the feeding choices made by ticks can dramatically influence the spread of disease in wildlife communities. I investigated the feeding behaviors of the American dog tick, *Dermacentor variabilis*, through a population genetic analysis. I hypothesized that if American dog ticks exhibit non-random host associations, then any genetic loci contributing to tick specialization should show strong associations with particular hosts. I assessed this hypothesis by collecting ticks directly from host animals, extracting DNA and generating high-throughput sequencing libraries only from legs (<100ng DNA per tick), characterizing many single nucleotide polymorphisms (SNPs), and comparing the genotypes of ticks from different host species. Using the fixation index (F_{ST}), a measure of population genetic subdivision, I found two loci with large allele frequency differences between ticks collected on different hosts. This suggests a host-structured tick population with non-opportunistic feeding and host specialization. This finding has consequences for the spread of pathogens in American dog tick and host animal populations.

INTRODUCTION

Vector borne disease transmission involves complex interactions between pathogens, host animals, and vectors. Ticks act as vectors for many pathogens, which they spread during feeding events. Ticks have multiple feeding life stages and feed on a

wide diversity of host species (Childs & Paddock, 2003; Paddock & Childs, 2003; Sonenshine, 1991). While much is understood about the off-host behaviors of ticks, relatively little is known about whether (or how) ticks evolve to specialize on their host animals.

Ticks are often assumed to be generalists, feeding on any of the available hosts within their host ranges (LoGiudice, Ostfeld, Schmidt, & Keesing, 2003; McCoy, Léger, & Dietrich, 2013). However, there is evidence that some presumably generalist ticks specialize on their hosts (Dodds, Martell, & Yescott, 1969; T. M. Kollars et al., 2000). This non-opportunistic feeding may be difficult to detect, especially when the host community composition is not measured. Specialization may exist at the species level, with all ticks sharing the same host preferences. Alternatively, there may be variation in preference within tick populations such that subpopulations specialize on different host species. In this case, surveys of feeding ticks will erroneously suggest generalist feeding behavior even when community composition is also measured.

The distinction between population-level and individual-level foraging behavior is epidemiologically relevant because generalists and specialists contribute unequally to disease transmission and infection when not all host species are capable of transmitting tick-borne pathogens. If ticks feed more frequently on the host animals most susceptible to disease, then disease prevalence may increase. Additionally, if tick populations are subdivided by host species, the tick subpopulations feeding on susceptible host animals may be at greater risk for infection than other tick subpopulations (McCoy, Léger, & Dietrich, 2013).

Non-random patterns of host association may be driven by variation among host species, which differ in habitat use, activity, body size, morphology, and grooming behaviors (Brunner & Ostfeld, 2008). If stable over time, host differences may impose

selective pressures on ticks to evolve non-opportunistic feeding patterns. Population-level foraging behavior can be observed by measuring tick burden on hosts, but determining if ticks feed non-randomly also requires data on host community structure and diversity, which are difficult to collect. I use an alternative means of assessing foraging behavior that focuses on the unique natural history of ticks. All ticks in the metastriate lineage, which includes dog ticks and other important disease vectors, mate on their adult-stage hosts (Sonenshine, 1991). This constrains the available mates for each tick to only those on the same host individual at the same time and links mate availability to host choice (Sonenshine1991). I hypothesize that if American dog ticks exhibit non-random host associations, then any genetic loci contributing to tick specialization should show strong associations with particular hosts.

Studies of population genetic structure in other tick species offer general support for the hypothesis that ticks have non-random host associations. These prior studies calculate genome- and locus-wise fixation indexes (F_{ST}) for microsatellite loci from ticks collected from different host animal species. F_{ST} describes the partitioning of genetic variation between and within populations, where high values represent a high fraction of variation partitioned uniquely within subpopulations, and low values indicate low differentiation with variation is shared between subpopulations.

Genotyping of microsatellite loci in the generalist tick *Ixodes ricinus* shows low but statistically significant population subdivision with genome-wide F_{ST} estimates ranging from 0.003 (comparing bird vs. rodent hosts) to 0.028 (comparing roe deer vs. wild boar hosts) (Kempf et al., 2011; McCoy, Léger, & Dietrich, 2013). Interestingly, ticks in the genus *Ixodes* do not mate on their host animals, unlike Metastriate lineage ticks, indicating that host-correlated population structure can arise under numerous life history strategies. The tick *Rhipicephalus (Boophilus) microplus* has a narrow host range

than *I. ricinus*, but shows a similar pattern of weak but significant subdivision by host species (cattle and deer) across 6 microsatellite loci genotyped (average F_{ST} = 0.029). When analyzed individually, F_{ST} estimates suggest 2 of the 6 microsatellites are significantly differentiated (F_{ST} = 0.06 and F_{ST} = 0.03) and may drive the genome-wide pattern observed (De Meeûs, Koffi, Barré, de Garine-Wichatitsky, & Chevillon, 2010).

I search for signs of host animal correlated population genetic structure using a single-nucleotide polymorphism (SNP) dataset instead of the more traditional microsatellite datasets employed by previous researchers. I adapted the double-digest restriction-site associated DNA sequencing (ddRADseq) method to obtain a large set of single nucleotide polymorphisms (SNPs) from small amounts of tick leg tissue (Peterson, Weber, Kay, Fisher, & Hoekstra, 2012). This procedure improves upon microsatellite genotype techniques by examining a wider subset of the genome, increasing the power to detect weak values of F_{ST}. I use the American dog tick as the study system for this work. The American dog ticks is epidemiologically important as a vector of *Rickettsia rickettsii*, and has an extensive host range of at least 50 vertebrate species (Bishopp & Trembley, 1945; Brillhart, Fox, & Upton, 1994). I hypothesize that American dog ticks will show patterns of population structure correlated with host identity. I collected adult American dog ticks directly from, raccoons (*Procyon lotor*) and Virginia opossums (*Didelphis virginiana*), two of their most common host species (Bishopp & Trembley, 1945; Cohen et al., 2010). These species differ in activity patterns, habitat use, body size, and grooming behaviors in ways that may result in differential survival and reproduction of American dog ticks. I assess the hypothesis that the American dog tick population structure reflects non-random association of ticks with raccoons or opossums by calculating F_{ST} for the SNP sites sampled across the American dog tick genome.

METHODS

Study site & tick collection

I collected ticks directly from live-trapped raccoons and opossums to assess whether tick populations are genetically structured by these host species. Field work was done at Caddo Lake National Wildlife Refuge (Karnack, TX) during June and July 2012. Caddo Lake National Wildlife Refuge is situated on the western side of Caddo Lake and contains habitats ranging from upland hard- and soft-wood forests to bottomland old growth hardwood forests. Raccoons and opossums were collected on two 1km long transects in riparian corridors located approximately 3km apart. Ten wire mesh traps (Tomahawk traps, incl order info) were placed on each transect at 100 m intervals. The first trapping transect was positioned in the riparian corridor of Harrison Bayou, which drains into Caddo Lake (UTM E0394485 N3614547). The second trapping transect was positioned along Goose Prairie, a slough that forms the western-most arm of Caddo Lake (UTM E0395684 N3618339). Animals were anesthetized with a combination of ketamine and dexmeditomidine (dosages in Appendix A). All animal work was done under the guidance and approval of The University of Texas at Austin Institutional Animal Care and Use Committee (IACUC) under protocol #AUP-2011-00057.

DNA Extraction From Tick Legs

Typical tick DNA extraction procedures involve grinding whole ticks (Halos et al., 2004; Hill & Gutierrez, 2003). I prepared the ddRADseq libraries with DNA extracted tick legs only. This minimized potential contamination with tick microbiota that would be present in a whole-tick DNA extraction, while still providing sufficient tissue for DNA isolation.

Adult American dog ticks were rinsed in 10% bleach followed by molecular grade water to remove surface contaminants. Legs were removed with sterile forceps and placed in a sterile 1.5 ml microcentrifuge tube. Each sample was frozen in liquid nitrogen and crushed with a sterile pestile. Crushed samples were extracted using the E.Z.N.A. Mollusk DNA extraction kit (Omega BioTek, Norcross, GA) following the manufacturer's instructions.

Tick Species ID Confirmation

American dog ticks were initially identified visually, and species identities were subsequently confirmed by sequencing of the 16S rRNA gene. An approximately 300 bp segment of the 16S rRNA gene was PCR amplified following the protocol of Black & Piesman, 1994, using primers 16S +1 (5'-CTG CTC AAT GAT TTT TTA AAT TGC TGT GG-3') and 16S -2 (5'-TTA CGC TGT TAT CCC TAG AG-3'). The PCR reaction mix consisted of 1.5mM $MgCl_2$, 200µM dNTPs (Fermentas), 1X Taq buffer (Thermo Scientific), 0.5U Taq (Thermo Scientific), 0.5µM 16S +1 primer, 0.5µM 16S -2 primer, and 2µL template DNA in a 25µL reaction. Thermal cycling conditions were 8 min denaturation at 94C, followed by 10 cycles of 1 min denaturation at 92C, 1 min annealing at 48C, and 1.5 min extension at 72C. An additional 32 cycles were done with 1 min denaturation at 92C, 1 min annealing at 54C, and 1.5 min extension at 72C. A final extension was done for 10 min at 72C, followed by a hold at 4C. PCR products were visualized on a 1.2% agarose gel, sequenced using an ABI 3730 DNA Analyzer with BigDye Terminator chemistry (Applied Biosystems), and compared to 16S rRNA sequences from tick species on GenBank to confirm species identity.

Reduced-Representation DNA Sequencing by ddRADseq

I identified single nucleotide polymorphisms in American dog ticks using ddRADseq, a technique for sequencing many small fragments across the entire genome. I created ddRADseq libraries from tick leg DNA following the procedure described by Peterson et al., 2012, and sequenced the DNA on the Illumina HiSeq platform.

Preliminary analysis of fragment size distributions after digesting tick DNA with *Eco*RI-HF and *Sph*I-HF (performed using a High-sensitivity DNA kit for 2100 Bioanalyzer, Agilent Technologies) suggested that approximately 3% of the American dog tick genome could be sequenced by targeting fragments 380-450bp in length. I digested tick leg DNA extracts with restriction enzymes *Eco*RI-HF and *Sph*I-HF (NEB, incl. order info) for 3 hours at 37^{O}C. I performed enzymatic cleanups with AMPure XP beads (Beckman-Coulter) following the manufacturer's protocol. Cleaned, digested DNA for each tick was quantified using a Pico-Green assay (Life Sciences), normalized to a total mass of DNA (either 20-30ng or 50-60ng) and ligated to DNA oligos containing compatible restriction overhangs and unique, inline (non-index read) sample barcodes (Appendix C). Ligated samples were pooled, cleaned again with AMPure XP beads and DNA fragments between 456-526bp (380-450bp genomic inserts plus 76bp of barcode and adapter sequence) were extracted using a Pippin Prep (2% agarose gel cartridge, Sage Science, Beverly, MA). Illumina index-read barcodes were added to pooled, post size-selection libraries by PCR, and finally the libraries were cleaned with DynaBeads (Life Techologies, Grand Island, NY) to increase the proportion of fragments flanked by both restriction sites.

I recovered a sufficient mass of DNA (>20ng) to perform ddRADseq on 105 ticks (74 from raccoons, 31 from opossums, and 4 excluded due to low DNA recovery). The 105 ticks were represented in three ddRAD libraries: one used samples with 20-30ng of

total pre-ligation DNA (low-concentration), and two used 50-60ng pre-ligation DNA per sample (high-concentration). Libraries were sequenced on one Illumina HiSeq2500 lane using paired-end, 100bp read chemistry with an index read at the University of Texas Genome Sequencing and Analysis Facility.

Sequence Mapping & Analysis

The raw data for each sequenced fragment consisted of two, 100bp reads ('read 1' and 'read 2'). Raw read 1 and read 2 sequence data were demultiplexed and quality filtered in Stacks v1.20 (Catchen, Hohenlohe, Bassham, Amores, & Cresko, 2013; Catchen, Amores, Hohenlohe, Cresko, & Postlethwait, 2011). Stacks retains sequences with intact barcodes and restriction sites with sliding window PHRED scores over 10. No reference genome exists for the American dog tick, and attempts to map sequences to the genome of a related tick, *Ixodes scapularis* failed due to the high divergence between these species. Consequently, I created a *de novo* assembly of all sequences. In order to use data from both 'read 1' and 'read 2' ends, I concatenated the quality-filtered reads to form 200bp-long single reads for *de novo* assembly in Stacks v1.20.

Preliminary analysis of the *de novo* assembly using Stacks v1.20 suggested a large number of paralogous sequences in the American dog tick genome, as evidenced by the prediction of over 100 concatenated reads with at least 10 unique haplotypes. The Stacks software (as used here) considers only a single individual's sequences when assigning genotypes, and does not use population-wide sequence information to resolve ambiguous genotypes. As a consequence, false-positive polymorphisms within individual paralogs are retained in the dataset, so the large number of paralogs may be a result of erroneous individual genotypes. I circumvented this process by using the *de novo*

assembly and software that determines the genotype likelihoods of individuals by comparing sequence variation across many individuals.

The quality filtered reads from individual samples were mapped to the consensus *de novo* reference genome using BWA-MEM (Li & Durbin, 2010; Li, 2013). Genotype likelihoods were calculated from the mapped sequences using the mpileup function of SAMtools (Li et al., 2009), which uses a population-aware likelihood-based algorithm. The numerous paralog sequences in the data set should be represented by only a few consensus sequences in the *de novo* reference. Using relatively relaxed mapping stringency, a diversity of paralog sequences will map to the same areas of the *de novo* reference, resulting in low likelihood scores for genotypes at those loci and ultimate exclusion from the data set.

The resulting likelihood data are converted to Variant Call Format (VCF) using VCFtools (Danecek et al., 2011) is used to perform a final quality filtering step and call bi-allelic genotypes. I included only SNPs that met the following criteria in subsequent analysis: (1) minor allele frequency of at least 10% across all sampled ticks, (2) minimum mean sequencing depth of 20X across the entire tick sample population, (3) minimum genotype quality score of 25, and (4) site sequenced in at least 75% of samples in each population.

I compared the allele frequencies in American dog ticks found on raccoons to the allele frequencies of American dog ticks recovered on opossums to look for evidence of population differentiation. The allele counts for the final SNP data were tabulated using VCFtools and analyzed in BayeScan (Foll & Gaggiotti, 2008). BayeScan estimates F_{ST} for each SNP and tests the hypothesis that F_{ST} estimates are outliers indicating either local adaptation (high F_{ST} values) or purifying selection (low F_{ST} values) against the null hypothesis that all estimates are consistent with genetic drift alone (Beaumont and

Balding 2004). The BayeScan model describes F_{ST} in terms of locus-specific (α) and population-specific (β) parameters, which are are estimated using a reversible-jump MCMC procedure, The corresponding values of F_{ST} and the locus-specific parameter (α) for the best-fitting model are reported for each locus, and the probability of observing those estimates under a drift-only model is reported. By estimating locus-specific and population-specific parameters, BayeScan is able to test for evidence of selection at each locus: if the α (locus-specific) parameter estimate is significantly greater than 0 for a locus, this is evidence for selection and the F_{ST} estimate is considered an outlier (Foll & Gaggiotti, 2008). Full details of the pipeline are in Appendix D.

I used the sequences of loci with outlier F_{ST} SNPs to conduct *blastn* searches for sequence homologs in other more fully sequenced species (Altschul, Gish, Miller, Myers, & Lipman, 1990). Outlier loci were separated back into their original, un-concatenated 'read 1' and 'read 2' ends, and each read was compared separately to other known sequences in GenBank.

RESULTS

Tick Collection & Processing

I collected 75 adult American dog ticks from 15 raccoons (mean tick burden = 5 ticks/animal) and 35 ticks from 3 Virginia opossums (mean burden = 11 ticks/animal) for a total of 110 ticks (Figure 3.1). Animals were found on both transects.

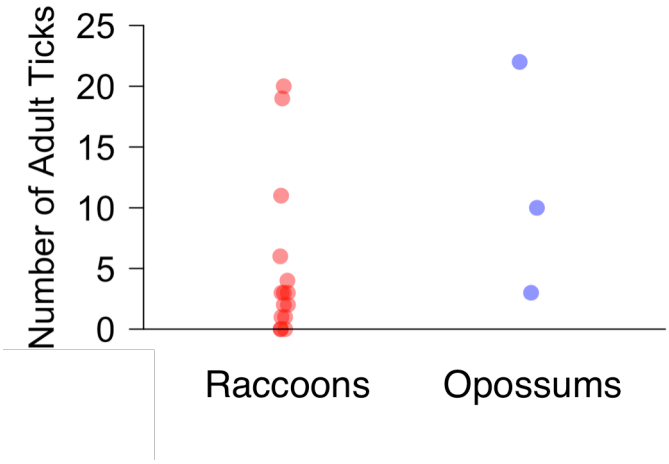

Figure 3.1: Tick burdens by host animal.
Number of adult American dog ticks sampled from raccoons (n=15) and opossums (n=3). Points are jittered to show overlap.

Sequence Mapping & Analysis

I identified 1,982 polymorphic sites that were present in at least 75% of samples across both populations and met read depth, quality, and minor allele frequency requirements. The F_{ST} values calculated by BayeScan range from 0.004 to 0.05 (mean F_{ST}=0.005). There was one statistically significant F_{ST} outlier locus (FST=0.05, q=0.03) with a private allele in ticks collected from raccoons, and one marginally significant F_{ST} outlier (FST=0.03, q=0.07) that showed the opposite host pattern—increased allele frequency in ticks on opossums (Figure 3.2). Allele frequencies for the outlier loci and a representative non-outlier locus are presented in Figure 3.3.

Read 1 of the largest outlier locus (F_{ST} = 0.05) shares 80% sequence identity and 60% query coverage with an *Ixodes scapularis* putative Na^{+}/Ca^{2+} exchanger mRNA (E-

value = $1x10^{-5}$; accession XM_002413690). The marginally significant sequence did not have any matches in GenBank.

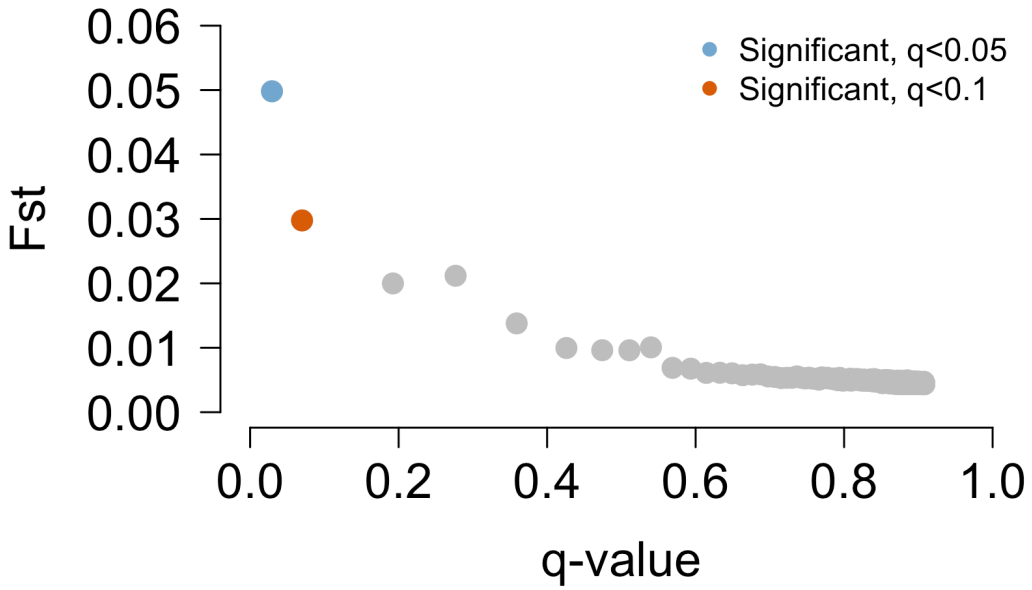

Figure 3.2: BayeScan F_{ST} outlier analysis results.
Each point represents a polymorphic site. F_{ST} values are estimated by comparing allele frequencies for variable sites in ticks collected from opossums and ticks collected from raccoons.

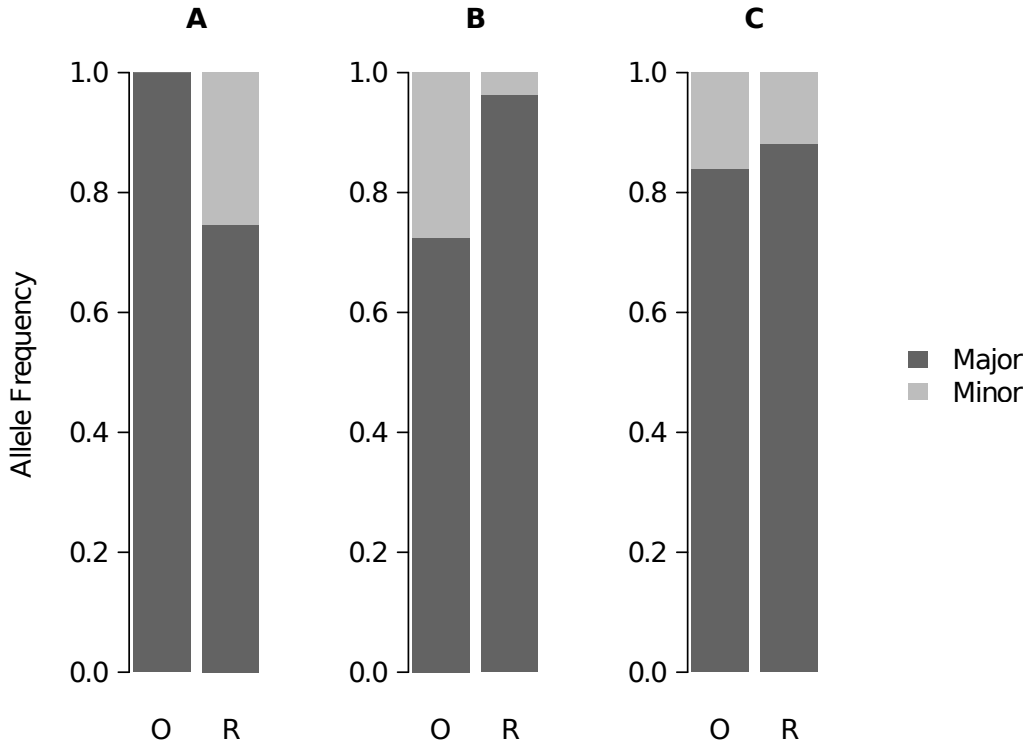

Figure 3.3: Allele frequencies.

Allele frequencies for opossums ticks (O) and raccoon ticks (R) for (A) the significant outlier locus with F_{ST} = 0.05, q = 0.03 (opossum ticks n=27, raccoon ticks n=57), (B) the marginally significant outlier locus with F_{ST} = 0.03, q = 0.07 (opossum ticks n=29, raccoon ticks n=66), and (C) a representative non-significant locus with F_{ST} = 0.004 q = 0.91 (opossum ticks n=31, raccoon ticks n=67).

DISCUSSION

High values of F_{ST} for two loci in the American dog tick genome indicate non-random association of these ticks with raccoons and opossums. This observation is consistent with several possible and non-mutually exclusive mechanisms by which ticks and hosts may non-randomly associate. Ticks may express host preference, may encounter opossums and raccoons with different frequencies, or may experience differential survival on these animals. Any of these mechanisms may produce local

adaptation to different host species within subpopulations of the American dog tick population consistent with the F_{ST} values observed. Comparison of allele frequencies of American dog ticks from raccoons and opossums revealed a genome-wide F_{ST} average of 0.005, suggesting a well-mixed population. However, two loci had significantly higher F_{ST} values of 0.05 and 0.03 than expected given the distribution of F_{ST} values across the genome. These results are consistent with other published studies reporting tick population structuring by host species.

The locations of these loci in the American dog tick genome are unknown. No reference genome exists for the American dog tick, and efforts to map sequence data to the divergent reference genome of *I. scapularis* were largely unsuccessful. However, the significant outlier locus shared strong sequence identity/similarity with a putative Na^+/Ca^{2+} exchanger mRNA from *I. scapularis*. Na^+/Ca^{2+} exchangers are found in a variety of cell types and are generally involved in mediating action potentials and maintaining charges in excitable cells. Reduced representation genome sequencing techniques like ddRADseq are intended to detect loci that are in linkage disequilibrium with sites truly under selection. It is possible that the American dog tick ortholog of the *I. scapularis* Na^+/Ca^{2+} exchanger may be linked to a site that is under selection due to pressures exerted by host identity.

If American dog tick adults feed non-randomly there are potential consequences for the spread of pathogens. American dog ticks take a single blood meal at each of their three feeding life stages (larvae, nymphs, and adults), and pathogens can be transmitted between ticks and hosts at each stage. Adult host associations are not necessarily predictive of larval or nymphal host associations. Previous studies report larval and nymphal ticks commonly parasitizing smaller animals, while adults are associated with larger host animals (Childs & Paddock, 2003). If host preferences are stable across the

tick lifespan, then disease prevalence would be higher in tick subpopulations favoring the disease reservoir. This would also place the preferred hosts of infected American dog tick subpopulations at greater infection risk. Conversely, if preferences vary with the life stage of the tick or manifest only during the adult stage, disease prevalence in tick populations would not correlate highly with host identity. The interaction between tick life cycle and pathogen spread has not been extensively studied, but warrants further attention.

The pattern of different foraging strategies within tick populations is supported by genetic data American dog ticks (this study) and *I. ricinus* (Kempf et al., 2011; McCoy, Léger, & Dietrich, 2013), and even ticks with narrower host ranges (such as *R. boophilus*) appear to associate non-randomly with hosts (De Meeûs et al., 2010). There are many other tick species with large host ranges and presumably generalist foraging behavior. If associations with hosts are truly non-random and have a reproductively isolating effect on ticks, this has far-reaching implications for the epidemiology and control of tick-borne pathogens. Cutting edge DNA sequencing techniques make the acquisition of large multi-locus genetic datasets with the power to detect weak signals of population structure inexpensive and easy to obtain. These tools should be applied to a diversity of tick species of epidemiological importance to test hypotheses about population structure and host associations.

Appendix C: ddRADseq Library Preparation Barcodes

Sample ID	Inline Barcode	Illumina Barcode	No. seqs recovered w/inline barcode
R9T8	TCGAT	CAGATC	430137
R9T7	AGCTA	CAGATC	1504408
R9T18	AACCA	CAGATC	586937
R9T12	CGGTA	CAGATC	2939263
R8T2	CTTGG	CAGATC	789307
R7T4	CTGTC	CAGATC	2051676
R7T3	GCTGA	CAGATC	1134622
R7T2	CGATC	CAGATC	530260
R7T17	GCCGT	CAGATC	922475
R7T14	GAGAT	CAGATC	1353119
R24T3	GGCCA	CAGATC	1821093
R24T2	GGCTC	CAGATC	1643537
R1T5	AATTA	CAGATC	688047
R1T4	CGTCG	CAGATC	1992613
R1T3	GAGTC	CAGATC	1214834
R1T2	CGTAC	CAGATC	1369784
R1T1	CTGAT	CAGATC	1877667
R14T3	GGTTG	CAGATC	577727
O6T9	GTAGT	CAGATC	1918154
O6T8	CATAT	CAGATC	2079668
O6T5	GGATA	CAGATC	911403
O6T3	CAACC	CAGATC	467588
O6T20	GTCCG	CAGATC	225236
O6T19	AAGGA	CAGATC	1221419
O6T18	CGAAT	CAGATC	1167138
O6T15	CGGCT	CAGATC	2091589
O6T12	ACACA	CAGATC	568114
O6T10	ACGGT	CAGATC	714969
O2T6	ATTAC	CAGATC	4689942
O2T3	CTGCG	CAGATC	1888102
O2T1	TGCAT	CAGATC	1002850
O25T2	GACAC	CAGATC	1116098
R22T3	GCATG	CAGATC	2537

Table C.1: Combinatoric Barcodes, 30ng Library

Sample ID	Inline Barcode	Illumina Barcode	No. seqs recovered w/inline barcode
R9T4	TGCAT	ACTTGA	1033501
R9T17	CTTGG	ACTTGA	1125397
R9T16	ATGAG	ACTTGA	2108014
R9T15	CAACC	ACTTGA	1028490
R9T14	GGCTC	ACTTGA	1504660
R9T11	GACAC	ACTTGA	1081119
R9T10	GCTGA	ACTTGA	1927463
R9T1	CGGTA	ACTTGA	1005881
R8T4	ACACA	ACTTGA	1257011
R8T3	ACGGT	ACTTGA	2029776
R8T1	CGATC	ACTTGA	627192
R7T9	GCATG	ACTTGA	340367
R7T6	GAGAT	ACTTGA	915074
R7T20	AGCTA	ACTTGA	728859
R7T13	AATTA	ACTTGA	1480534
R7T11	CTGCG	ACTTGA	1011330
R7T10	ACTTC	ACTTGA	1104008
R7T1	GGATA	ACTTGA	1562186
R24T4	ACTGG	ACTTGA	1266148
R24T1	CTGTC	ACTTGA	1502406
R23T2	CGTCG	ACTTGA	1512340
R1T6	TCGAT	ACTTGA	498092
R1T11	ATACG	ACTTGA	1334035
R1T10	GGCCA	ACTTGA	1635778
R17T1	GCCGT	ACTTGA	1282470
R10T2	GAGTC	ACTTGA	805512
O6T22	CGTAC	ACTTGA	1150465
O6T21	CGGCT	ACTTGA	1138409
O6T17	CTGAT	ACTTGA	1783389
O6T16	GGTTG	ACTTGA	1030332
O6T14	AAGGA	ACTTGA	682112
O6T11	CGAAT	ACTTGA	1160719
O2T8	AACCA	ACTTGA	545668
O2T10	CATAT	ACTTGA	1573871
R9T19	ATTAC	ACTTGA	6571

Table C.2: Combinatoric Barcodes, 60ng Library 1

Sample ID	Inline Barcode	Illumina Barcode	No. seqs recovered w/inline barcode
R9T9	CGGTA	GATCAG	811699
R9T6	CTGTC	GATCAG	1440321
R9T5	GCATG	GATCAG	480144
R9T3	ACACA	GATCAG	747266
R9T2	ACGGT	GATCAG	605287
R9T13	CAACC	GATCAG	457022
R7T7	CTGAT	GATCAG	1001363
R7T5	GAGAT	GATCAG	1415975
R7T19	GGCCA	GATCAG	1037021
R7T18	AAGGA	GATCAG	644084
R7T16	ATACG	GATCAG	1894491
R7T12	CTTGG	GATCAG	1444079
R23T1	CGTAC	GATCAG	1486181
R22T5	CGTCG	GATCAG	1157045
R22T4	GAGTC	GATCAG	1176117
R22T2	ATTAC	GATCAG	1195563
R1T9	AACCA	GATCAG	385646
R1T7	GGATA	GATCAG	1509325
R17T3	CGAAT	GATCAG	930605
R15T3	ACTGG	GATCAG	859967
R15T2	TGCAT	GATCAG	1296028
R15T1	CATAT	GATCAG	774097
R14T1	GCCGT	GATCAG	1259186
R12T1	GCTGA	GATCAG	1194318
O6T7	GGTTG	GATCAG	492529
O6T6	AGCTA	GATCAG	932593
O6T2	TCGAT	GATCAG	297413
O6T13	CGGCT	GATCAG	1202186
O6T1	GGCTC	GATCAG	680228
O2T9	ACTTC	GATCAG	533004
O2T7	GACAC	GATCAG	1497445
O2T4	ATGAG	GATCAG	966420
O25T3	CTGCG	GATCAG	1136898

Table C.3: Combinatoric Barcodes, 60ng Library 2

Appendix D: ddRADseq Analysis Pipeline

Software documentation & downloads

1. Stacks 1.20: http://creskolab.uoregon.edu/stacks/
2. BWA 0.7.7: http://bio-bwa.sourceforge.net/
3. samtools 0.1.19: http://samtools.sourceforge.net/
4. VCFtools 0.1.12b: http://vcftools.sourceforge.net/
5. BayeScan 2.1: http://cmpg.unibe.ch/software/BayeScan/
6. R 3.0.1: http://www.r-project.org/

Analysis Pipeline

1. Demultiplex & quality-filter libraries (Stacks v1.20)

```
process_radtags -P -p /raw/ -o /output_dir -b
     /barcodes_file --inline_index --renz_1 sphI --renz_2
     ecoRI -q -r -i gzfastq
```

2. Concatenate quality-filtered paired reads (bash)

Two separate bash scripts are used to generate the concatenated data. The first pastes corresponding lines of *R1.fq and *R2.fq files together with a tab between the read sequences, and the second removes tabs from the file to create a single string of sequence data. Read 2 is not reverse-complemented.

```
for R1 in *R1.fq
do
     Rname=`echo $R1 | sed 's/.fq\+//'`
     R2=${R1%R1.fq}R2.fq
     #echo $R1, $R2
     paste $R1 $R2 > $Rname.concat.fq
done

for R in *concat.fq
do
     cat $R | sed 's/[ \t]//g' >> $R.untabbed.fq
done
```

3. *de novo* Data Assembly (Stacks v1.20)

```
denovo_map.pl -m 10 -n 2 -b 1 -S -t -D "job name" -o
     /output_dir -O /population_map
     -s /path/to/data/sample1/concatenated_read/
     -s /path/to/data/sample2/concatenated_read/
```

4. Build a pseudo-reference from the *de novo* assembled genome (R v3.0.1)

The short R script below converts the consensus *de novo* assembly, found in batch_x.catalog.tags.tsv, into a fasta file that can be used for downstream analysis.

```
     setwd('path/to/denovo/output/)
     # Import the data and check the structure
     tags<-read.table('batch_1.catalog.tags.tsv',
header=FALSE)
     # all the sequences are in $V9 and the locus IDs are
in $V3
     # each sequence needs a fasta header
     fa.id<-paste('>', tags$V3, '_arbitrary_info', sep='')
     fa<-cbind(fa.id, as.character(tags$V9))
     # use '\n' as the separating character to get fasta
headers and sequences to alternate lines
     write.table(fa, file=psuedoreference.fa', quote=FALSE,
sep='\n', row.names=FALSE, col.names=FALSE)
```

5. Index psuedoreference genome (BWA 0.7.7)

```
bwa index /path/to/psuedoreference.fa
```

6. Map quality-filtered sequences (output of step 1) to pseudoreference (BWA 0.7.7)

Each sample is mapped with a separate call to BWA. An example line is below:

```
bwa mem -M -R
     "@RG\tID:sample1\tPL:Illumina\tLB:sample1\tSM:sample1"
     /path/to/psuedoreference.fa
     /path/to/sample1.concat.fq.untabbed.fq > sample1.sam
```

7. Convert *.sam files to *.bam files; sort and index *.bam files

Example commands for a single sample:

```
samtools view -F 4 -b -S -o sample1.bam sample1.sam
samtools sort sample1.bam sample1.sorted
samtools index sample1.sorted.bam
```

8. Call genotypes with mpileup (SAMtoos v0.1.19)

```
samtools mpileup -DuIf /path/to/psuedoreference.fa -C50
     /path/to/all/samples/*.sorted.bam >
     pseudoref_mapped_genotypes.bcf
```

9. Final quality filter and allele counts (bcftools/vcftools v0.1.12b)

First convert the bcf file to a vcf file:

```
bcftools view -v -c -g pseudoref_mapped_genotypes.bcf >
     pseudoref_mapped_genotypes.vcf
```

Then filter the entire dataset for minor allele frequency, coverage depth and quality, with output as a new .vcf file:

```
vcftools --vcf /path/to/pseudoref_mapped_genotypes.vcf --
     maf 0.1 --min-meanDP 20 --minGQ 25 --recode --out
     /output/path/pseudoref_filtered_maf0.1_minmeanDP20_min
     GQ25
```

Finally, output allele frequencies separately for the two sub-populations:

```
vcftools --vcf
     /path/to/pseudoref_filtered_maf0.1_minmeanDP20_minGQ25
     .recode.vcf --keep /path/to/keep_raccoons.txt --max-
     missing 0.75 --counts --out
     /output/path/pseudoref_raccoon_counts_pop_filtered_max
     missing0.75
vcftools –vcf
     /path/to/pseudoref_filtered_maf0.1_minmeanDP20_minGQ25
     .recode.vcf --keep /path/to/keep_opossum.txt --max-
     missing 0.75 --counts --out
     /output/path/pseudoref_opossum_counts_pop_filtered_max
     missing0.75
```

10. Convert allele frequency outputs into BayeScan format (R v3.0.1)

The script is designed to interactively process allele frequency data for two populations only. The script outputs two text files, one for each population, in the format specified by BayeScan. However, BayeScan takes a single input file, so these files must be merged manually (simply copy and paste into a single text file). Header lines must also be added to ensure the files will be properly loaded by Bayescan.

11. Conduct outlier analysis test (BayeScan v2.1)

Consult the BayeScan documentation for further details on how to run BayeScan and interpret its outputs.

```
/install/path/for/BayeScan2.1/source/bayescan_2.1
     /path/to/Bayescan_Input_file -od /output/path/ -
     out_freq
```

Chapter 4: A mathematical model predicting the role of host preference in the transmission of *Ehrlichia chaffeensis*

ABSTRACT

Tick-borne pathogens cause numerous cases of human disease each year when ticks acquire infection from wildlife and subsequently feed on humans. The lone star tick, *Amblyomma americanum*, feeds on a wide diversity of wildlife species including mammals such as white-tailed deer, raccoons, opossums, small mammals, birds, and reptiles. Lone star ticks transmit the bacterial pathogen *Ehrlichia chaffeensis* within white-tailed deer populations. Other wildlife species are not susceptible and transmission of *E. chaffeensis* cannot occur with lone star ticks feed on other wildlife species. This observation has led to the prediction that wildlife communities with many hosts species that are not susceptible to infection may have lower prevalence of tick-borne pathogens when ticks feed opportunistically. However, the true feeding preferences of lone star ticks are not well understood, and if lone star ticks have strong feeding preferences for certain hosts then observed *E. chaffeensis* prevalence may be in part explained by lone star tick foraging preference. I estimated lone star tick feeding preference by developing a mathematical model of *E. chaffeensis* transmission in a multi-host wildlife community and fitting the model to data on *E. chaffeensis* prevalence. The model suggests that lone star ticks have low preference for white-tailed deer, but that preference for white-tailed deer is stronger when deer are less abundant than alternative hosts. The model also reveals great uncertainty in the probability of transmission of *E. chaffeensis* from deer to ticks. These results underscore the importance of considering both community structure and tick foraging behavior when conducting tick-borne disease prevalence studies.

INTRODUCTION

Ehrlichia chaffeensis is an emerging tick-borne pathogen that causes a significant public health burden. Approximately 1,100 people were infected with *E. chaffeensis* in the United States in 2012 (Adams et al., 2014). People infected with this pathogen experience fever and increased susceptibility to secondary infections, and severe infections may be fatal in approximately 4.9% of cases (Bakken, 1996; Dumler & Bakken, 1995). These infections occur when people are bitten by lone star ticks (*Amblyomma americanum*) that have previously fed upon infected animals. Up to 10% of adult lone star ticks may be infected in *E. chaffeensis* endemic areas, but not all populations of lone star ticks are infected (Mixson et al., 2006). Lone star ticks acquire *E. chaffeensis* infection from feeding on infected white-tailed deer, which are the only definitively established reservoir host for *E. chaffeensis* (Lockhart et al., 1997). Disease prevalence in white-tailed deer populations can be as high as 25% (Yabsley et al., 2004).

White-tailed deer are the only known reservoirs for *E. chaffeensis*, but they are not the only vertebrate animals parasitized by lone star ticks (Childs & Paddock, 2003). In fact, lone star ticks have an exceptionally wide host range and have been documented not only on mammals, but on birds and reptiles as well (Bishopp & Trembley, 1945; Durden, Oliver, & Kinsey, 2001; T. M. Kollars et al., 2000). Serological surveys of raccoons, coyotes, and foxes, which are common lone star tick host animals, show these animals have antibodies reactive with *E. chaffeensis* (Davidson et al., 1999; Paddock & Childs, 2003; Yabsley et al., 2008). This is consistent with natural exposure to this pathogen, and indicates these animals have been parasitized by infected ticks. There is no evidence that these animals become actively infected with *E. chaffeensis*, or that they can transmit the pathogen to susceptible ticks. When infected ticks feed on hosts that cannot

become infected, no transmission can occur. These epidemiological dead-ends can have profound consequences for disease transmission.

The spread of tick-borne pathogens such as *E. chaffeensis* may be tempered by these bites on non-reservoir animals, particularly because ticks like the lone star tick feed only three times during their life. Lone star ticks have three life stages (larva, nymph, and adult), and consume one blood meal during each stage (Sonenshine, 1991). Infected female lone star ticks cannot pass *E. chaffeensis* to their offspring, so no larvae are infected (Long et al., 2003). Lone star ticks have at most two opportunities to transmit *E. chaffeensis* to a competent reservoir host, assuming they acquire infection during the larval feeding. If the diversity of available hosts is large and if ticks use a generalist or opportunistic foraging strategy, there may be few chances for ticks to spread *E. chaffeensis* to spread in wildlife communities.

The observation that host range and wildlife community diversity interact to drive prevalence of tick-borne diseases is often phrased as the "dilution hypothesis" (LoGiudice, Ostfeld, Schmidt, & Keesing, 2003). Non-reservoir host animals lower overall disease prevalence by absorbing bites that might otherwise occur on competent reservoirs. The classic case for disease dilution is *Borrelia burgdorferi*, the causative agent of Lyme disease, which is carried by the deer tick (*Ixodes scapularis*). *Borrelia burgdorferi* has multiple vertebrate reservoirs that vary in their competence as reservoirs (Gray, 1998). Some reservoir animals, like the white-footed mouse (*Peromyscus leucopus*) are easily infected and can readily transmit infection to ticks. These highly competent reservoirs are heavily parasitized by nymphal *I. scapularis*, providing an opportunity for infection early in the life of the tick. Therefore, white-footed mice contribute greatly to disease prevalence, particularly when they are at high relative abundance. When white-footed mice are in lower relative abundance than their less

competent counterparts, endemic *B. burgdorferi* infection persists but at a much lower prevalence (LoGiudice, Ostfeld, Schmidt, & Keesing, 2003).

Does *E. chaffeensis* prevalence respond to increases in species richness and reservoir relative abundance in the same manner as *B. burgdorferi*? The predictions are not straightforward. The standard dilution hypothesis built around the *B. burgdorferi* example assumes that the tick vector is a true generalist, but different foraging strategies may produce different results (McCoy, Léger, & Dietrich, 2013). For example, if lone star ticks prefer to feed on white-tailed deer, the competent *E. chaffeensis* reservoir, increased biodiversity of potential mammalian hosts may have little effect on infection prevalence. Conversely, preference for non-reservoir hosts may accelerate dilution in more diverse communities.

There is anecdotal evidence that lone star ticks have some host preferences, despite their wide host range. Several studies note that larval lone star ticks feed more frequently on smaller animals than on white-tailed deer (T. Kollars, 1993). However, white-tailed deer seem to have particularly high nymphal and adult lone star tick burdens as compared to other host animals (though these observations are not controlled for by host animal body size, which may influence tick burdens). Raccoons and coyotes are also regarded as preferred hosts for lone star ticks (Childs & Paddock, 2003).

The evidence for preference is typically presented in terms of number of ticks parasitizing hosts, with more commonly parasitized hosts assumed to be preferred. But this definition lacks ecological context; as the dilution hypothesis research on deer ticks indicates, the availability of hosts is an important determinant of tick burdens. If ticks encounter and parasitize hosts randomly, then more common hosts are encountered and parasitized more frequently by chance and not by preference. I define lone star tick

preference for a host animal for the purposes of this paper as a tick burden greater than expected given the relative abundance of that host animal in the wildlife community.

Jointly studying community structure and foraging preferences in natural tick populations is challenging, but mathematical models can be used to describe these interactions. I constructed a compartmental mathematical model of *E. chaffeensis* transmission in a wildlife population with both reservoir hosts and alternative hosts. I use the model to estimate key epidemiological parameters under a variety of different host relative abundance scenarios. My model improves upon previous compartmental and agent-based models of lone star tick population and disease transmission dynamics that consider only single host species (Gaff, Gross, & Schaefer, 2009; Gaff & Gross, 2007; Haile & Mount, 1987; Wang, Grant, & Teel, 2012). I use this model to evaluate the hypothesis that lone star ticks have host preferences that impact disease transmission. I specifically look for evidence that lone star ticks parasitize white-tailed deer more often than alternative hosts by fitting the model to empirical data.

The primary objective of the model fitting process is to estimate preference of lone star ticks for white tailed deer. The model includes a preference parameter describing the probability with which ticks feed on white-tailed deer or alternative hosts, Ticks encounter white-tailed deer and alternative hosts in proportion to the relative abundance of those host types, and preference describes the probability with which a tick will choose to feed on the encountered host type. Alternative hosts are ecologically equivalent to white-tailed deer in all regards except their ability to serve as reservoirs for *E. chaffeensis* infection. I calculated the endemic equilibrium disease prevalence (percentage of population infected) predicted by the model for different parameter sets and evaluate those predictions against estimates of *E. chaffeensis* prevalence in the southeastern United States using a Bayesian Markov Chain Monte Carlo (MCMC).

A secondary objective of the model fitting process is to estimate the probability of transmission of *E. chaffeensis* from ticks to deer, and from deer to ticks. Most of the core epidemiological parameters of the model are derived from published reports on the transmission dynamics and course of infection for *E. chaffeensis* (Davidson et al., 2001; Dawson et al., 1994; Ewing et al., 1995; H. D. Gaff & Gross, 2007; Haile & Mount, 1987; Sonenshine, 1991; Varela-Stokes, 2007; Wang et al., 2012). These parameters include the duration of infection in ticks and white-tailed deer, and the recovery rate and duration of immunity for deer. The probability of transmission is critical to describing disease dynamics, but typically the most difficult epidemiological parameter to estimate. Experimental transmission studies are possible, but few have been done for *E. chaffeensis*, ticks, and white-tailed deer. Given this uncertainty, I also estimated transmission probabilities jointly with preference as part of the model fitting MCMC.

In the following sections, I describe the process by which preference data were obtained from the literature and summarized using meta-analysis, the parameterization of the mathematical model, the model fitting process, and ultimately the estimated preference and transmission probabilities that best describe the pattern of *E. chaffeensis* prevalence in the southeastern United States.

METHODS

There are four distinct steps in this model fitting process: (1) describe natural variation in disease prevalence using meta-analysis, (2) build a compartmental disease model that realistically captures disease dynamics, (3) parameterize that model (with the exception of preference and transmission rate) using values derived from previously published studies, and (4) use Bayesian Markov Chain Monte Carlo (MCMC) to estimate

parameters by comparing prevalence outputs of the compartmental model to the natural variation described by the meta-analysis. The specific details of these steps are outlined in the following sections.

Disease prevalence meta-analysis

The southeastern United States has a well-documented history of *E. chaffeensis* infection in tick and white-tailed deer populations. I focused on a four-state section including Florida, Georgia, North Carolina, and South Carolina (Figure 4.1). I gathered reports of *E. chaffeensis* prevalence in adult tick and white-tailed deer populations in these states published between 1973 and 2005 (Appendix E) and used these prevalence values to estimate an expected distribution of disease prevalence values. These studies measured infection status of adult lone star ticks by PCR amplification of either 16S rRNA or the variable-length PCR target (VLPT). While published reports of prevalence in nymphal ticks exist, the species identities of the nymphs are not reported and so these studies were not included.

Studies that reported information on PCR and serological testing of white-tailed deer peripheral blood were used to infer prevalence in white-tailed deer populations. PCR testing for *E. chaffeensis* in white-tailed deer used the 16S rRNA target. Serological detection of *E. chaffeensis* infection was performed by either indirect fluorescent antibody testing (IFA) or enzyme-linked immunosorbant assays (ELISAs). There are reports of antibody reactivity in raccoons, but no reports of PCR positive animals (Yabsley et al., 2008). These data are excluded from the analysis because the role of these animals in transmission is not clear. Antibody reactivity suggests prior exposure, but without evidence from experimental transmission studies to confirm a role in

transmission, it is possible that these animals have natural exposure but do not serve as reservoirs.

Estimates of disease prevalence from these studies are variable, with no one study completely representative of the entire region. I used the individual prevalence estimates (PCR prevalence in lone star ticks, PCR prevalence in white-tailed deer, and antibody prevalence in white-tailed deer) to develop expected distributions of disease prevalence. I used a Bayesian multinomial logistic regression to generate posterior prevalence distributions. The multinomial logistic regression was performed in R version 3.0.2 using the package BayesLogit (Polson, Scott, & Windle, 2013; R Core Team, 2013).

SI-SIRS model of *Ehrlichia chaffeensis* transmission

I developed a compartmental model of *E. chaffeensis* transmission, which explicitly describes the full life cycle of lone star ticks, including off-host and on-host ticks. The model includes white-tailed deer as reservoir hosts, and alternative hosts that are not a reservoir for the pathogen (Figure 4.2). Disease dynamics differ for ticks and white-tailed deer: in ticks, once infected they remain infected until death; in contrast, white-tailed deer can recover from infection and acquire immunity. Over time, the immunity wanes and the deer become susceptible again. These dynamics are described in the model as "susceptible-infected" (SI) for ticks, and "susceptible-infected-recovered-susceptible" (SIRS) for the white-tailed deer. The full SI-SIRS model is solved numerically to determine the equilibrium state, and the resulting equilibrium infection prevalences are used for further analysis. The model is solved in Python using SciPy and NumPy packages (Jones, Oliphant, Peterson, & Al., 2001-; van der Walt, Colbert, & Varoquaux, 2011). Model equations can be found in Appendix F.

Ehrlichia chaffeensis is spread between ticks and white-tailed deer during tick feeding events. Questing (host-seeking) ticks feed on white-tailed deer with probability ϕ_D, where ϕ_D represents host preference expressed as the probability a tick will feed on a deer if encountered. Questing ticks express preference for alternative hosts by feeding on them with probability $(1-\phi_D)$ if encountered. Encounter between ticks and white-tailed deer is proportional to the relative abundance of the host animals, where W is the size of the deer population, R is the size of the alternative host population, and H is the size of the total host population $(W+R)$. Therefore the number of ticks feeding on white-tailed deer is $\phi_D W/H$, and the number of ticks feeding on alternative hosts is $(1-\phi_D)R/H$. The value of ϕ_D is unknown, and its estimation using MCMC methods is one of the primary objectives of this project.

The size of the tick population depends on the number of hosts in the community and the total number of ticks each host can support. Tick populations were tracked both on- and off-host to accurately reflect the fact that ticks spend most of their lives off-host. Because ticks never recover from infection, the susceptible population can only be replenished by the birth of ticks. I imposed a carrying capacity on ticks both on- and off-host, and include birth and death parameters to capture the natural life cycle of the ticks. Population sizes for white-tailed deer and alternative hosts are fixed. The susceptible deer population is replenished by the loss of immunity in previously infected deer.

Parameter estimates

Animals move between the model compartments as they encounter infected individuals, contract infection, and (in the case of white-tailed deer), recover from infection and eventually lose immunity. Birth of new, susceptible ticks into the system is critical to accurately capturing realistic disease dynamics. Four key parameter types are

necessary to describe these dynamics: transmission probability, recovery rate, loss of immunity rate, and (for ticks), birth and death rates. Most parameter values for the model are taken from previously published reports. Transmissibility and preference are estimated (Table 4.1). A full list of model parameters and their values can be found in Appendix E. The derivation of the model parameters from published reports is outlined in the following sections.

Symbol	Parameter
ϕ_W	tick preference for deer
ρ_{TD}	deer to tick pathogen transmission rate
ρ_{DT}	tick to deer pathogen transmission rate

Table 4.1: Parameters estimated in MCMC

Transmission

I also used the Bayesian MCMC to estimate transmission probability, and compare those estimates to values from the literature. One study considered experimental infection of deer using infected ticks, though the authors do not report how many infected ticks were used to inoculate each deer (Jaworski, Bowen, & Wasala, 2013). Four naïve white-tailed deer were each exposed to 30 ticks, but the prevalence of infection in those tick populations was not known. Two of the four deer became infected with *E. chaffeensis*. In the most extreme case that all 30 ticks on each deer were infected, the probability of infection from a single bite is 2%. Assuming at least one of the 30 ticks on each deer is infected, the probability of infection is 50%. Averaging over those two scenarios, the rough estimate of the probability of tick to deer *E. chaffeensis* probability is 26%. Another study attempted to infect nymphal lone star ticks by placing them on a deer

that had been innoculated with *E. chaffeensis*. After feeding and molting to the adult stage, the study reports 6% of the ticks were infected with *E. chaffeensis* (Varela-Stokes, 2007).

Both these studies advance understanding of the physiology of transmission but have experimental designs that do not lend to easily estimating natural transmission probabilities. However, they suggest that transmission probabilities are asymmetric, and that tick to deer transmission probability is higher than deer to tick transmission probability. Given that these are single point estimates that are not corroborated by other studies, these transmission values are not included in the model. Instead, transmission probabilities from ticks to deer ($\rho_{T->D}$) and from deer to ticks ($\rho_{D->T}$) are estimated jointly along with ϕ_D using MCMC model fitting.

Recovery & Waning Immunity

Parameterizing recovery rate and the rate at which immunity is lost in white-tailed deer requires some simplifying assumptions about the course of *E. chaffeensis* infection in these animals. I assumed for the purposes of this model that deer are infectious from the moment of exposure. Because the sensitivity and specificity for *E. chaffeensis* PCR detection assays have not been determined, I further assumed that all infectious deer will test positive for *E. chaffeensis* by PCR. These assumptions allow me to use the duration which an animal is PCR positive to describe the length of infection. Similarly, I assumed that animals with antibody reactivity to *E. chaffeensis* are either infectious or recovered. Once antibody reactivity is lost, animals are assumed to once again be susceptible to *E. chaffeensis* infection. The duration for which an animal remains antibody-positive at a titer of 1/64 reflects the duration of immunity. The reciprocal of the average duration

infected is the recovery rate (λ), and the reciprocal of the average duration antibody positive is the rate at which immunity is lost (ν) (Appendix E). All rates are per week.

Other Model Parameters

Birth rates, host-finding times, feeding times, carrying capacities on- and off-host, and death rates were used to describe the life cycle of ticks in the model. These parameters and their citations are listed in Appendix E.

Model fitting

MCMC Overview

I used the SI-SIRS model, parameterized with values derived from published reports on *E. chaffeensis* infection dynamics and tick demography, to infer preference of lone star ticks for white-tailed deer (ϕ) and transmission probabilities from tick to deer ($\rho_{T->D}$) and deer to tick ($\rho_{D->T}$). Parameters are inferred using MCMC where new parameters (ϕ, $\rho_{T->D}$, and ρ_{D-T}) are proposed for the SI-SIRS model, the equilibrium state of the system of equations is solved numerically for that parameter set, and the likelihood of the model given empirical prevalence distributions derived from the meta-analysis is calculated.

New parameters are proposed in blocks from pilot independent normal distributions ($\theta_j \sim N(x_j, \sigma)$) where θ_j is the value of new parameter j, x_j is the most recently accepted value of parameter j, and σ is fixed at 0.05 for all parameters. The prior distribution for each parameter is $U(0,1)$ and proposals are accepted or rejected using a Metropolis algorithm. The likelihood function is described in greater detail in the following section. This process is repeated for 10,000 iterations to form the basis of a multivariate normal proposal distribution $N(\overline{X}_i, \Sigma)$, where $\overline{X}_i$ is a vector of mean parameter values from the pilot run and Σ is the parameter covariance matrix from the

pilot run. New parameters proposals are drawn from this distribution for an additional 110,000 iterations.

Likelihood Function

The previously described meta-analysis produced expected distributions of *E. chaffeensis* prevalence in adult ticks and white-tailed deer shown in Figure 4.3 and described more fully in the results section. These prevalence distributions were based on the fraction of ticks PCR-positive for *E. chaffeensis* infection, the fraction of white-tailed deer PCR-positive for *E. chaffeensis* infection, and the fraction of white-tailed deer antibody-positive for *E. chaffeensis*. I use the logit-transforms of these three distributions for the likelihood function.

The observed data (PCR or antibody positivity) can be related to the model compartments S, I, or R. Equilibrium infection prevalence in adult ticks in the model is compared to the PCR prevalence of *E. chaffeensis* in adult ticks from the meta-analysis. Equilibrium infection prevalence in white-tailed deer is likewise compared to PCR prevalence of *E. chaffeensis* in white-tailed deer as described by the meta-analysis. Antibody positivity has a less straightforward interpretation. Infected and recovered white-tailed deer can both be antibody positive, and many studies of *E. chaffeensis* prevalence in white-tailed deer conduct only serological surveys. This makes it impossible to determine what fraction of deer are naturally infected or recovered. This overlap is reflected in the likelihood calculation used; the equilibrium numbers of infected and recovered deer are summed and compared to the antibody prevalence described by the meta-analysis.

The likelihood of a parameter set θ_j is calculated as

$$L\left(\theta_j\right) \sim \prod P(\theta_j \mid N\left(\bar{Y}_k, \sigma_k\right), \quad (1)$$

where $N(\bar{Y}_K, \sigma_k)$ is the distribution of prevalence values for population component k.

Sensitivity to host population sizes and relative abundance

The number of available host animals and the relative abundance of reservoir hosts has the potential to influence the values of the parameters estimated with the MCMC. The relative abundance of white-tailed deer to non-reservoir species is unknown and likely variable across the study sites from which prevalence data were obtained. I explored the influence of host relative abundance on model predictions by changing the number of hosts available. Three different relative abundance schemes were used: 200 deer and 400 alternative hosts (Scenario 1), 200 deer and 200 alternative hosts (Scenario 2), and 400 deer and 200 alternative hosts (Scenario 3). The on-host carrying capacity, the number of ticks that can feed on a single host animal at any one point in time, was held constant at 100 ticks/animal for all runs.

RESULTS

Disease Prevalence Meta-Analysis

I found seven studies that met the criteria for inclusion in the meta-analysis . These studies reported a wide range of *E. chaffeensis* prevalence values for adult lone star ticks (28 unique estimates of infection by PCR ranging from 0-10.1%) and white-tailed deer (8 unique estimates of infection from by PCR ranging from 0-26.5%, and 12 unique estimates of exposure by antibody testing ranging from 30.8%-100%). These values were

used to estimate posterior distributions of disease prevalence (Figure 4.3). I estimated a median *E. chaffeensis* prevalence of 1.32% in adult lone star ticks (95% credible interval (CI) 0.664%-1.97%). In white-tailed deer populations I estimated 22.5% PCR prevalence (95% CI 5.04%-40.0%) and 64.2% antibody prevalence (95% CI 46.1%-82.4%).

Model fitting

All of the relative abundance scenarios produce outputs that yield prevalence values consistent with expectations from the literature (Figure 4.4). However, the different relative abundance scenarios give rise to different estimates for preference and transmission probability. MCMC chains can be found in Appendix H (Figures H.1, H.2, & H.3).

Preference

Preference marginal posterior probability distributions for all three relative abundance scenarios are shown in Figure 4.5, left column. When white-tailed deer are less common than alternative hosts (Scenario 1), preference is estimated to be a median 0.21 (95% CI 0.060-0.074). Preference for white-tailed deer is estimated to be lower in Scenario 2, where deer have the same abundance as alternative hosts (median preference = 0.044, 95% CI 0.023-0.11). Median preference for white-tailed deer in Scenario 3, where deer are more common than alterative hosts, is 0.040 (95% CI 0.014-0.21).

Transmission

The probability of transmission from ticks to deer is largely stable across all three relative abundance scenarios. Scenarios 1, 2 and 3 produce median tick to deer transmission of 0.022 (95%CI 0.0055-0.089), 0.055 (95% CI 0.018-0.15), and 0.040 (95% CI 0.0075-0.15), respectively (Figure 4.5, middle column). Median deer to tick transmission probability (Figure 4.5, right column) is 0.27 for Scenario 1 (95% CI 0.066-

0.90), 0.58 for Scenario 2 (95% CI 0.27-0.96), and 0.38 for Scenario 3 (95% CI 0.094-0.93).

Parameter Correlations

Transmissibility estimates are highly negatively correlated with estimates of preference for white-tailed deer in all relative abundance scenarios (Figure 4.6). The two transmission probabilities are themselves weakly positively correlated.

Tick Burdens on White-Tailed Deer

The model allows up to 100 ticks on each host animal (white-tailed deer or alternative). The average number of ticks parasitizing each animal for accepted posterior parameter set is far fewer than 100, and can be calculated from the on-host tick population sizes to produce posterior distributions of tick burden (Figure 4.7). When white-tailed deer are less common than alternative hosts (Scenario 1) they are more heavily parasitized by ticks with a median tick burden of 5.59 ticks/deer (95% CI 1.66-19.42 ticks/deer). In Scenario 2, where deer and alternative hosts are in equal relative abundance, the median deer burden is 2.11 ticks/deer (95% CI 1.12-4.98 ticks/deer). Finally, Scenario 3 (white-tailed deer more common) produces a median burden of 1.41 ticks/deer (95% CI 0.99-13.31 ticks/deer).

DISCUSSION

Are lone star ticks generalists or specialists?

The model describes tick-host interactions as a product of innate preferences and encounter rates. The preference parameter (ϕ_D) describes the probability that a tick feeds on white-tailed deer if those animals are encountered. Baseline encounter rates are proportional to host relative abundance. Values of ϕ_D greater than 0.5 indicate that ticks

encountering white-tailed deer feed on those animals more than 50%, consistent with specialization on deer. All relative abundance scenarios resulted in median ϕ_D estimates less than 0.5, and only in Scenario 1 (low white-tailed deer relative abundance) 95% CI include 0.5. These results are largely consistent with specialist foraging on alternative hosts instead of white-tailed deer.

Preference estimates are somewhat sensitive to the relative abundance of deer and alternative hosts. Preference for deer is predicted to be lower when more deer are present in the model system. The ϕ_D 95% CIs become wider as deer become less common in the model system, indicating less certainty about the value of preference.

Transmission-Preference Correlation

The estimated parameters ϕ_D, $\rho_{T->D}$, and $\rho_{D->T}$ are all highly correlated. Increases in the transmissibility of *E. chaffeensis* can compensate for low preference for deer to produce reasonable infection prevalence even when there are few deer available for ticks to parasitize. The model is not fully identifiable from these data, as multiple combinations of these parameters are equally likely. The non-identifiability of the model did not impede proper mixing of the MCMC chains, but the parameter correlations produce wide marginal posterior probability distributions. The correlations suggest an epidemiologically meaningful interaction between encounter rates (related to ϕ_D) and transmission probability. The preference parameter ϕ_D modulates the rate at which ticks encounter their hosts, such that high values of ϕ_D increase the encounter rate with deer, and low values decrease the encounter rate.

A wide range of transmission probabilities are feasible under different encounter rates, but encounter rates impose some constraints on the parameter estimates. High preference values coupled with lower transmission probabilities can theoretically produce

the same prevalence result as the low preference and high transmission probability scenario, but such parameter combinations are not well-supported by the model because contact rates between deer and infected ticks are much greater than between ticks and infected deer.

Infection prevalence in adult ticks is lower than in white-tailed deer. Deer have many contacts with ticks, so despite the low prevalence in tick populations, the force of infection experienced by deer may be greater (depending on the burden of ticks on each deer). A low tick-to-deer transmission probability coupled with a low tick preference for deer can counteract this force of infection to keep prevalence values within the expected range. Ticks, on the other hand, have at most three contacts with an infected deer (and perhaps far fewer depending on the availability of alternate hosts and the tick preferences). The model supports higher values of deer to tick transmission probability in order to achieve even the quite small infection prevalence values observed. In fact, the asymmetry of transmission probabilities is the exact opposite of what was estimated from the literature, where deer to tick transmission probabilities were suggested to be lower than tick to deer transmission probabilities.

Transmission probability is not likely to vary with ecological or behavioral change. Changes in relative abundance of hosts or differences in tick foraging behavior should not appreciably change the probability that *E. chaffeensis* is passed from a deer to a tick during feeding. The wide marginal posterior probability for deer to tick transmission suggests the model under all relative abundance scenarios suggests that additional empirical work is needed to fully characterize this parameter.

The sensitivity of the model dynamics and posterior parameter estimates to changes in relative abundance suggests that underlying community structure is a key aspect of *E. chaffeensis* epidemiology. While the importance of community structure in

driving the dynamics of tick-borne pathogen transmission has become widely acknowledged, many of the studies used in parameterizing the model and developing the likelihood function were conducted and published before the dilution hypothesis gained popularity as an explanation for variation in prevalence of tick-borne pathogens. Future field research on *E. chaffeensis* should incorporate community diversity studies. Knowing the relative abundance of host animals corresponding to observed disease prevalence will help with direct evaluation of the dilution hypothesis for *E. chaffeensis*, and will also increase the utility of this model in predicting foraging behavior or transmission probabilities. Alternatively, robust and repeatable laboratory studies on transmission probabilities could be used to re-parameterize the model and help refine estimates of host preference under different relative abundance scenarios.

The Importance of Host Relative Abundance in Disease Intervention

Decreases in the relative abundance of reservoir hosts are expected to lead to decreased prevalence of tick-borne diseases (LoGiudice, Ostfeld, Schmidt, & Keesing, 2003). However, the model results suggest that observed prevalence of *E. chaffeensis* can be explained equally well under several different reservoir relative abundance scenarios, given that tick host preferences are allowed to change. The model-based estimates of lone star tick preference for deer are sensitive to host relative abundance, indicating this is an important component of *E. chaffeensis* epidemiology that is currently poorly understood. Relative abundance of white-tailed deer, the only known reservoir of *E. chaffeensis*, is likely variable across the studies used in the model fitting process. If true relative abundance of white-tailed deer was known for each study site, it might be possible to infer a single, stable preference of ticks for host animals from the model.

Ehrlichia chaffeensis infection prevalence is highly variable across the southeastern United States, but the underlying source of that variation is not known. White-tailed deer are the only known reservoir of *E. chaffeensis*, and encounter rates between ticks and reservoir hosts are believed to be a function of host availability. Host relative abundance, and hence tick-host encounter rate, is likely a key source of variation. Different community compositions may give rise to different disease prevalence and risk, and these results may not necessarily follow the predictions made under the dilution hypothesis if tick preference for deer is sufficiently strong. If ticks feed non-opportunistically, their preferences may be exploited for targeted disease interventions.

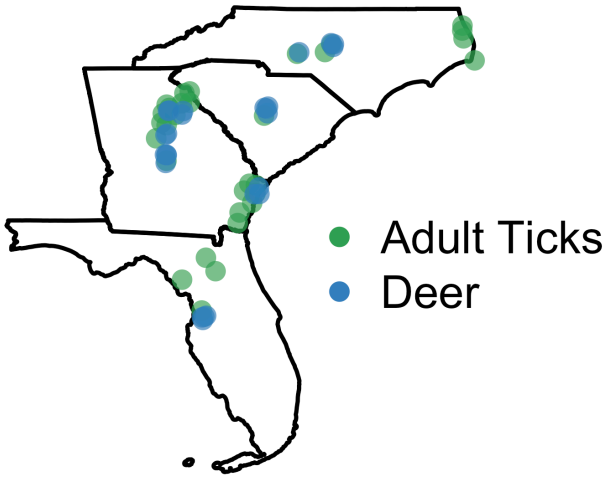

Figure 4.1: Study site locations.
Adult tick and white-tailed deer *Ehrlichia chaffeensis* reports were obtained from studies conducted in North Carolina, South Carolina, Georgia, and Florida.

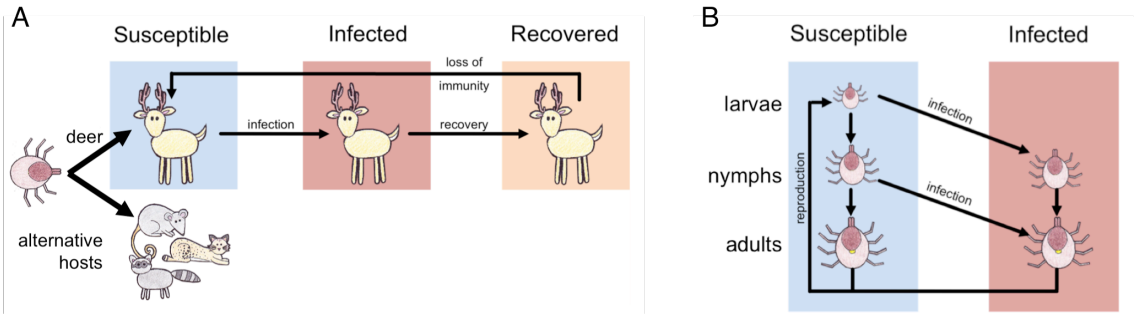

Figure 4.2: Conceptual model.
Ehrlichia chaffeensis infection follows SIRS dynamics in white-tailed deer (A) and SI dynamics in ticks (B).

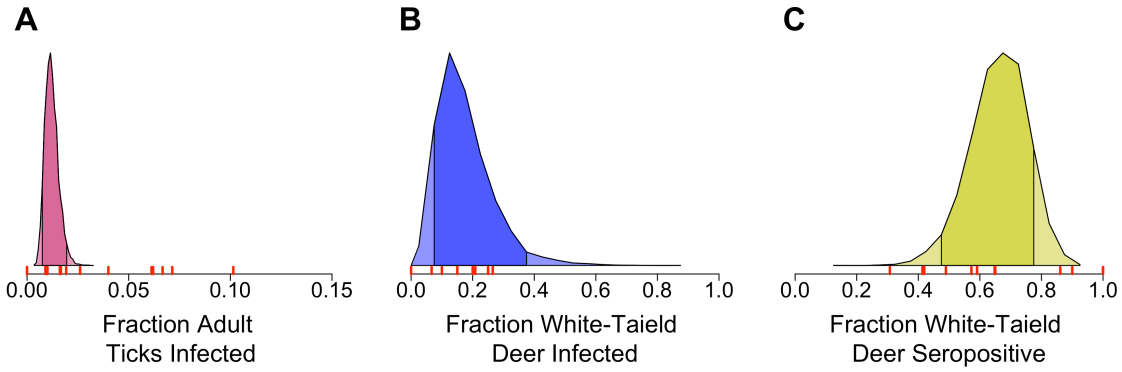

Figure 4.3: Disease prevalence meta-analysis results.
Posterior *Ehrlichia chaffeensis* PCR prevalence distributions for (A) adult ticks (n=28), (B) PCR prevalence for white-tailed deer (n=8), and (C) seroprevalence for white-tailed deer (n=12) are plotted with empirical data points (red points along x-axis).

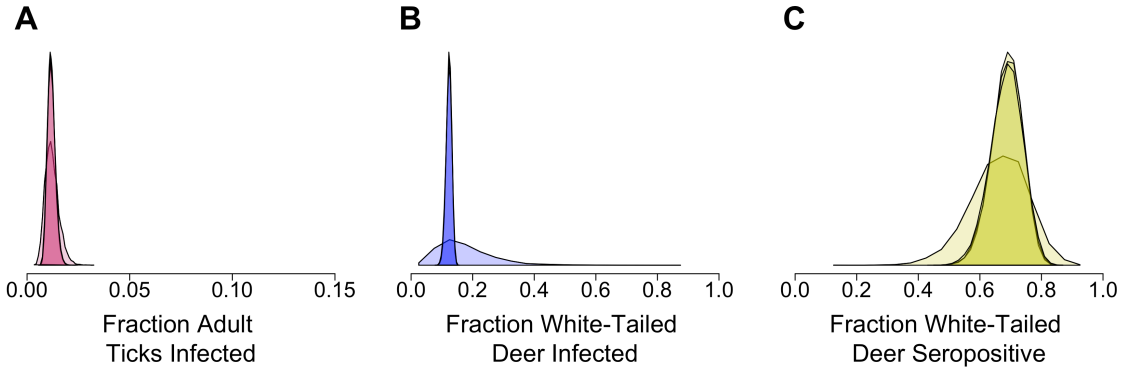

Figure 4.4: Model fit to empirical data.
Light colored curves represent the meta-analysis prevalence outputs as in Figure 4.3 (but with different y-axis scaling). The darker, narrower curves are the posterior prevalence outputs under each relative abundance scenario for (A) PCR prevalence in adult ticks, (B) PCR prevalence in white-tailed deer, and (C) seroprevalence in white-tailed deer. All relative abundance scenarios produce similar prevalence posteriors that are consistent with the meta-analysis results.

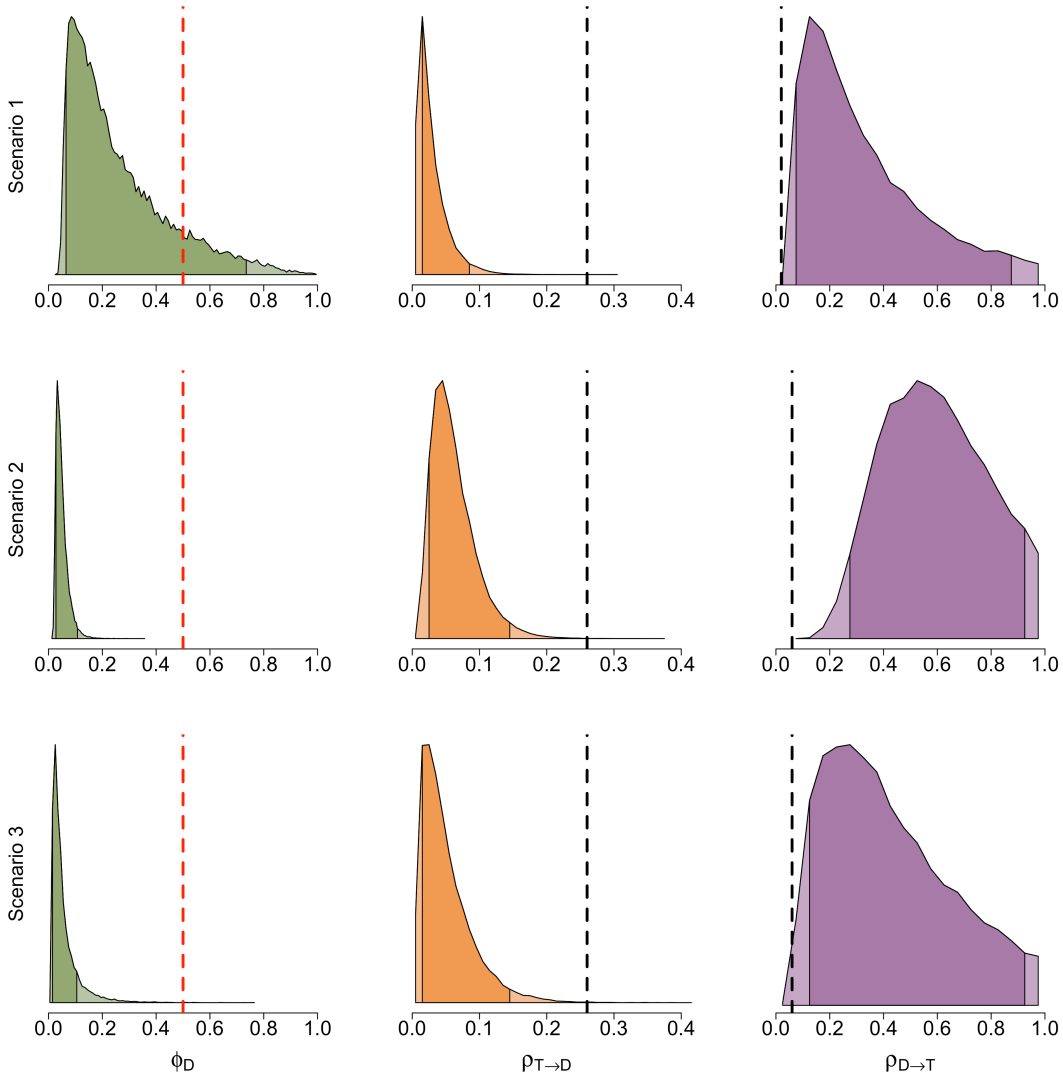

Figure 4.5: Marginal posterior probability densities for preference (ϕ_D), tick to deer transmission probability ($\rho_{T->D}$), and deer to tick transmission probability ($\rho_{D->T}$)

The three rows from top to bottom represent posterior parameter distributions for relative abundance scenario 1 (1 deer:2 alternative hosts), scenario 2 (1 deer:1 alternative host), and scenario 3 (2 deer:1 alternative host). The dashed red line for the ϕ_D posteriors represents the boundary between specializing on deer ($\phi_D > 0.5$) or on alternative hosts ($\phi_D < 0.5$). The dashed black lines for the $\rho_{T->D}$ and $\rho_{D->T}$ parameters represent the estimates for those parameters derived from the literature.

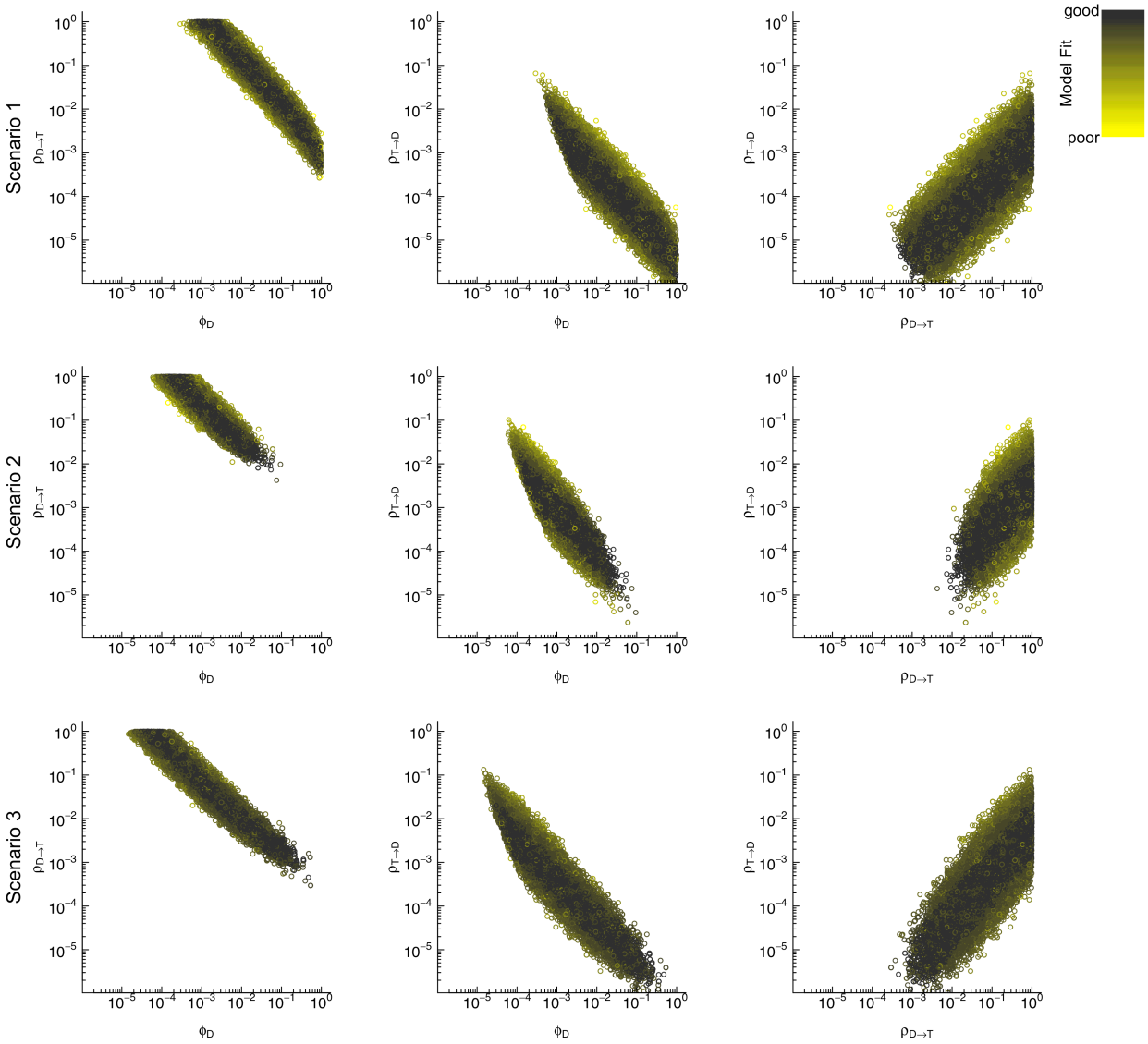

Figure 4.6: Parameter correlations for different relative abundance scenarios.
The three rows from top to bottom represent posterior parameter distributions for relative abundance scenario 1 (1 deer:2 alternative hosts), scenario 2 (1 deer:1 alternative host), and scenario 3 (2 deer:1 alternative host). Darker colors indicate higher posterior probability for those parameter sets.

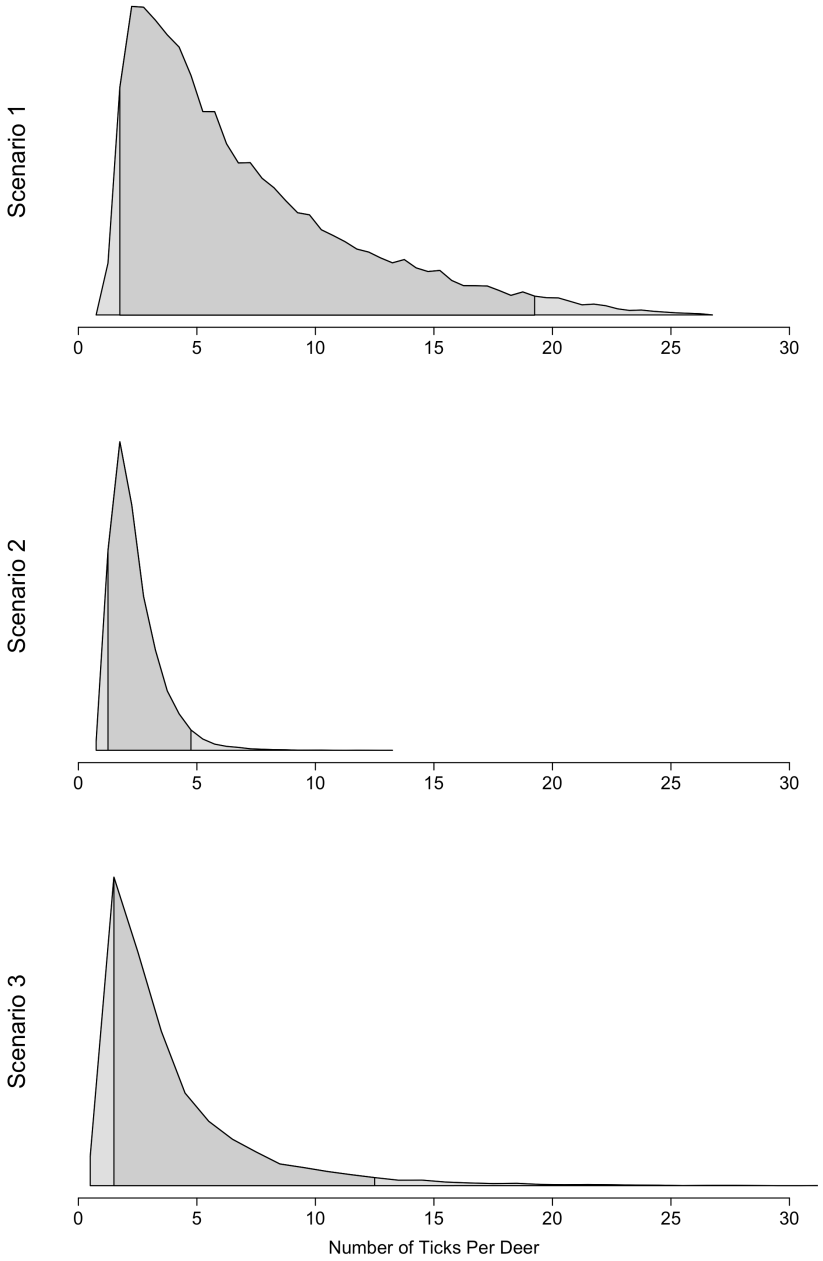

Figure 4.7: Tick burdens, white-tailed deer.
Each histogram is the posterior distribution of the average number of ticks of all life stages parasitizing deer under each relative abundance scenario. The darkened regions represent the 95% CIs for each scenario.

Appendix E: Disease Prevalence Data

Parameter	Test	Target	Sample Size	Prevalence	State	Year	Citation
adult tick infection	PCR	VLPT	14	7.1%	NC	2002	Mixon et al. 2006
adult tick infection	PCR	VLPT	15	6.7%	GA	2004	Mixon et al. 2006
adult tick infection	PCR	VLPT	22	0.0%	NC	2003	Mixon et al. 2006
adult tick infection	PCR	VLPT	29	0.0%	FL	2003	Mixon et al. 2006
adult tick infection	PCR	VLPT	41	0.0%	GA	2005	Mixon et al. 2006
adult tick infection	PCR	VLPT	42	0.0%	GA	2005	Mixon et al. 2006
adult tick infection	PCR	VLPT	48	0.0%	GA	2003	Mixon et al. 2006
adult tick infection	PCR	VLPT	49	4.1%	GA	2001	Varela et al. 2004
adult tick infection	PCR	VLPT	50	0.0%	FL	2003	Mixon et al. 2006
adult tick infection	PCR	VLPT	50	0.0%	GA	2002	Varela et al. 2004
adult tick infection	PCR	VLPT	54	0.0%	GA	2004	Mixon et al. 2006
adult tick infection	PCR	VLPT	55	0.0%	NC	2003	Mixon et al. 2006
adult tick infection	PCR	VLPT	59	0.0%	GA	2004	Mixon et al. 2006
adult tick infection	PCR	VLPT	60	1.7%	GA	2002	Mixon et al. 2006
adult tick infection	PCR	VLPT	62	1.6%	GA	2004	Mixon et al. 2006
adult tick infection	PCR	VLPT	69	10.1%	GA	2003	Mixon et al. 2006
adult tick infection	PCR	VLPT	72	0.0%	FL	2003	Mixon et al. 2006
adult tick infection	PCR	VLPT	74	0.0%	GA	2003	Mixon et al. 2006
adult tick infection	PCR	VLPT	76	0.0%	GA	2004	Mixon et al. 2006
adult tick infection	PCR	VLPT	79	0.0%	SC	2002	Mixon et al. 2006
adult tick infection	PCR	VLPT	82	0.0%	NC	2002	Mixon et al. 2006
adult tick infection	PCR	VLPT	104	1.0%	NC	2002	Mixon et al. 2006
adult tick infection	PCR	VLPT	104	1.9%	GA	2003	Mixon et al. 2006
adult tick infection	PCR	16S	111	0.9%	GA	not given	Whitlock et al. 2000
adult tick infection	PCR	VLPT	115	2.6%	NC	2002	Mixon et al. 2006
adult tick infection	PCR	16S	129	6.2%	GA	not given	Whitlock et al. 2000
adult tick infection	PCR	16S	151	0.0%	GA	not given	Whitlock et al. 2000
adult tick infection	PCR	VLPT	299	0.7%	GA	2003	Varela et al. 2004

Parameter	Test	Target	Sample Size	Prevalence	State	Year	Citation
deer infection	PCR	16S	5	20.0%	GA	1995	Little et al. 1998
deer infection	PCR	16S	5	0.0%	GA	1995 and 1996	Lockhart et al. 1997
deer infection	PCR	16S	10	10.0%	GA	1995 and 1996	Lockhart et al. 1997
deer infection	PCR	16S	20	15.0%	GA	1995 and 1996	Lockhart et al. 1997
deer infection	PCR	not specified	36	25.0%	FL	1983-2002	Yabsley et al. 2003
deer infection	PCR	not specified	60	6.7%	SC	1991-2001	Yabsley et al. 2003
deer infection	PCR	not specified	72	20.8%	NC	1982-2002	Yabsley et al. 2003
deer infection	PCR	not specified	98	26.5%	GA	1973-2002	Yabsley et al. 2003
deer antibody	IFA	recip. titer >=64	5	100.0%	GA	1995	Little et al. 1998
deer antibody	IFA	recip. titer >=64	10	90.0%	GA	1995 and 1996	Lockhart et al. 1997
deer antibody	IFA	recip. titer >=64	10	100.0%	GA	1995 and 1996	Lockhart et al. 1997
deer antibody	IFA	recip. titer >=64	20	65.0%	GA	1995 and 1996	Lockhart et al. 1997
deer antibody	IFA	recip. titer >=64	22	59.1%	NC	1991	Dawson et al. 1994
deer antibody	IFA	recip. titer >=64	26	30.8%	SC	1988; 1991-1992	Dawson et al. 1994
deer antibody	IFA	recip. titer >=64	36	86.1%	FL	1989 and 1991	Dawson et al. 1994
deer antibody	IFA	recip. titer >=128	104	41.3%	FL	1983-2002	Yabsley et al. 2003
deer antibody	IFA	recip. titer >=128	124	41.9%	SC	1991-2001	Yabsley et al. 2003
deer antibody	IFA	recip. titer >=128	131	57.3%	NC	1982-2002	Yabsley et al. 2003
deer antibody	IFA	recip. titer >=128	243	49.0%	GA	1973-2002	Yabsley et al. 2003
deer antibody	IFA	recip. titer >=64	301	64.8%	GA	1989-1991	Dawson et al. 1994

Table E.1: Prevalence Data

Appendix F: SI-SIRS Model Equations

Tick compartments; stage-structured; Susceptible-Infected (SI) dynamics

(1) questing larvae

$$A_S = GbK_T - A_S\phi_W K_W \frac{W}{H} - A_S(1-\phi_W)K_R \frac{R}{H} - A_S d_{Aq}$$

(2) feeding larvae (on deer)

$$B_{SW} = A_S\phi_W K_W \frac{W}{H} - B_{SW} r_B - B_{SW} d_{Bo}$$

(3) feeding larvae (on alternate hosts)

$$B_{SR} = A_S(1-\phi_W)K_R \frac{R}{H} - B_{SR} r_B - B_{SR} d_{Bo}$$

(4) questing nymphs (susceptible)

$$C_S = B_{SR} + \left(B_{SW} - B_{SW} r_B \rho_{D->T} \frac{W_I}{W} \right) - C_S\phi_W K_W \frac{W}{H} - C_S(1-\phi_W)K_R \frac{R}{H} - C_S d_{Cq}$$

(5) questing nymphs (infected)

$$C_I = B_{SW} r_B \rho_{D->T} \frac{W_I}{W} - C_I\phi_W K_W \frac{W}{H} - C_I(1-\phi_W)K_R \frac{R}{H} - C_I d_{Cq}$$

(6) feeding nymphs (susceptible; on deer)

$$D_{SW} = C_S\phi_W K_W \frac{W}{H} - D_{SW} r_D - D_{SW} d_{Do}$$

(7) feeding nymphs (susceptible; on alternate hosts)

$$D_{SR} = C_S(1-\phi_W)K_R \frac{R}{H} - D_{SR} r_D - D_{SR} d_{Do}$$

(8) feeding nymphs (infected; on deer)

$$D_{IW} = C_I\phi_W K_W \frac{W}{H} - D_{IW} r_D - D_{IW} d_{Do}$$

(9) feeding nymphs (infected; on alternate hosts)

$$D_{IR} = C_I(1-\phi_R)K_R \frac{R}{H} - D_{IR} r_D - D_{IR} d_{Do}$$

(10) questing adults (susceptible)

$$E_S = D_{SR} + \left(D_{SW} - D_{SW} r_D \rho_{D->T} \frac{W_I}{W} \right) - E_S \phi_W K_W \frac{W}{H} - E_S (1 - \phi_W) K_R \frac{R}{H} - E_S d_{Eq}$$

(11) questing adults (infected)

$$E_I = D_I + D_{SW} r_D \rho_{D->T} \frac{W_I}{W} - E_I \phi_W K_W \frac{W}{H} - E_I (1 - \phi_W) K_R \frac{R}{H} - E_I d_{Eq}$$

(12) feeding adults (susceptible; on deer)

$$F_{SW} = E_S \phi_W K_W \frac{W}{H} - F_{SW} r_F - F_{SW} d_{Fo}$$

(13) feeding adults (susceptible; on alternate hosts)

$$F_{SR} = E_S (1 - \phi_R) K_R \frac{R}{H} - F_{SR} r_F - F_{SR} d_{Fo}$$

(14) feeding adults (infected; on deer)

$$F_{IW} = E_I \phi_W K_W \frac{W}{H} - F_{IW} r_F - F_{IW} d_{Fo}$$

(15) feeding adults (infected; on alternate hosts)

$$F_{IR} = E_I (1 - \phi_W) K_R \frac{R}{H} - F_{IR} r_F - F_{IR} d_{Fo}$$

(16) replete adults (susceptible)

$$G_S = F_{SR} + \left(F_{SW} - F_{SW} r_F \rho_{D->T} \frac{W_I}{W} \right) - G_S d_{Ge}$$

(17) replete adults (infected)

$$G_I = F_{IW} + F_{SW} r_F \rho_{D->T} \frac{W_I}{W} - G_I d_{Ge}$$

White-tailed deer compartments; Susceptible-Infected-Recovered-Susceptible (SIRS) dynamics

(18) susceptible white-tailed deer

$$W_S = W_S \ln(1 - \rho_{T->D})(C_I + F_{IW}) + W_R \upsilon$$

(19) infected white-tailed deer

$$W_I = -W_S \ln(1 - \rho_{T->D})(C_I + F_{IW}) + W_I\lambda$$

(20) recovered white-tailed deer

$$W_R = W_I\lambda - W_R\upsilon$$

Appendix G: Model Parameters

Symbol	Parameter	Value	Citation
b	larval tick birth	10.75	Gaff and Gross 2007
r_B	on-host larval tick feeding rate	0.9	Sonenshine 1991
r_D	on-host nymphal tick feeding rate	0.9	Sonenshine 1991
r_F	on-host adult tick feeding rate	0.5	Sonenshine 1991
d_{Aq}	questing larval tick death rate	0.02	Haile & Mount 1987
d_{Bo}	on-host larval tick death rate	0.62	Wang et al. 2012
d_{Cq}	questing nymphal tick death rate	0.01	Haile & Mount 1987
d_{Do}	on-host nymphal tick death rate	0.69	Wang et al. 2012
d_{Eq}	questing adult tick death rate	0.001	Haile & Mount 1987
d_{Fo}	on-host adult tick death rate	0.21	Wang et al. 2012
d_{Ge}	engorged adult tick death rate	0.08	Haile & Mount 1987
λ	recovery rate	0.23	*see table G.3*
ν	rate of immunity loss	0.05	*see table G.3*

Table G.1: Constant parameters.
Parameter values are derived from data presented in the cited papers. All rates are calculated per week. Parameters derived from Haile & Mount 1987 used data on bottomland habitat.

Symbol	Parameter	Value
K_T	off-host carrying capacity for ticks	40,000
K_W	on-deer carrying capacity for ticks	100
K_R	carrying capacity, ticks on-alternative hosts	100
W	deer population size	200 or 400
H	total host population size (deer + alternative)	400 or 600
R	alternative host population size	200 or 400

Table G.2: Parameters varied for sensitivity analysis

Animal ID	Last day post-exposure PCR+ (λ)	Last day post-exposure AB+ (ν)	Citation
1	14		Ewing et al. 1995
2	31		Varela-Stokes 2007
3	31		Dawson et al. 1994
4	27		Dawson et al. 1994
5		108	Davidson et al. 2001
6	32	278	Davidson et al. 2001
7	45	73	Davidson et al. 2001
8		108	Davidson et al. 2001
average durations (weeks)	4.29	20.25	
Inverse average duration (weeks)	**0.23**	**0.05**	

Table G.3: Raw data for estimating white-tailed deer λ and ν.

λ (recovery rate) and ν (rate of immunity loss) are estimated from experimental infection time-series data. Shaded cells represent measurements not taken in the associated study.

Appendix H: MCMC Chains

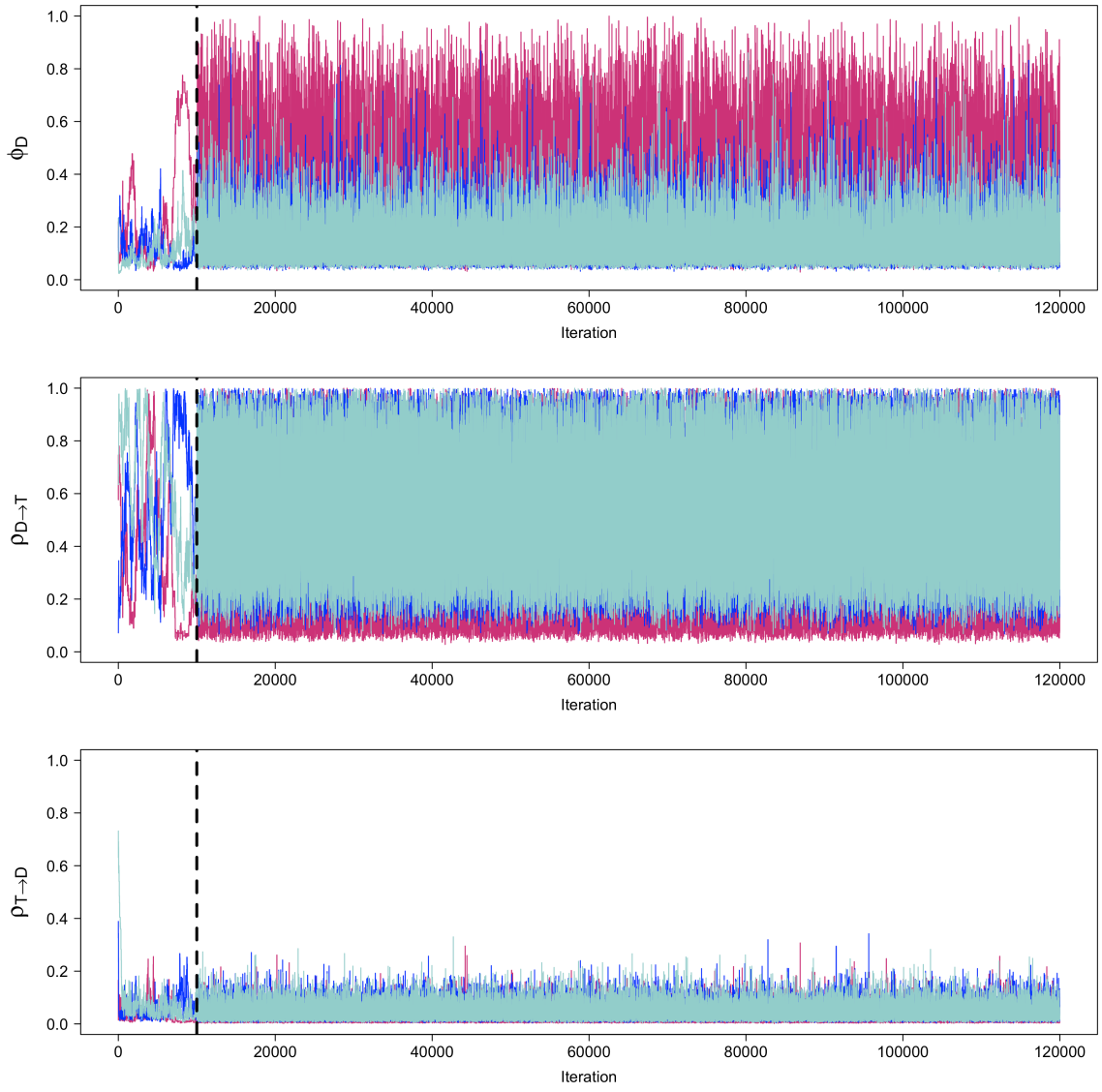

Figure H.1: Scenario 1 relative abundance chains.
Different colors represent chains seeded by different parameter values. Chains prior to the dashed black line were from pilot iterations used to generate the multivariate normal proposal distribution used for iterations 10,000 and onward.

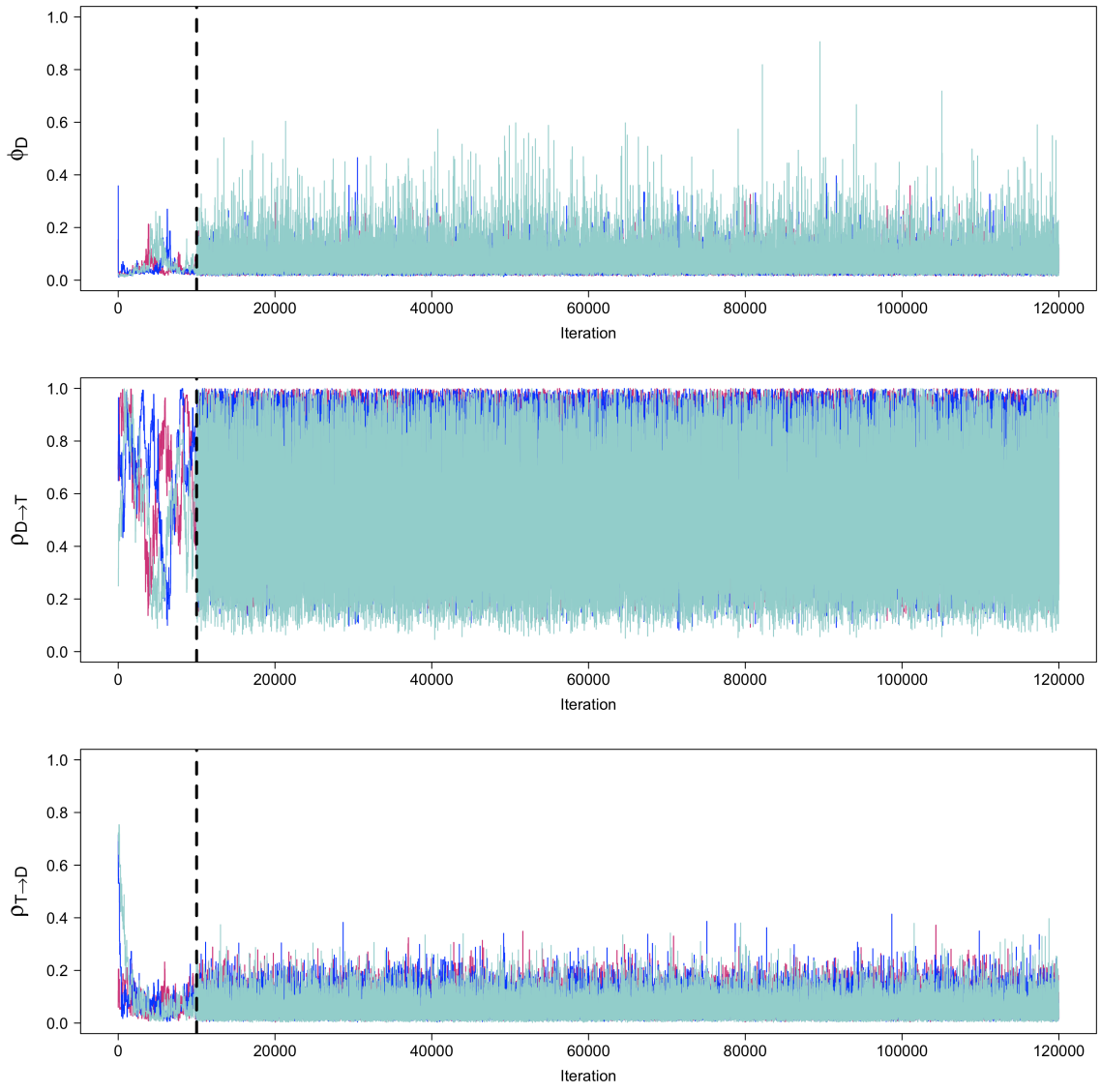

Figure H.2: Scenario 2 relative abundance chains.
Different colors represent chains seeded by different parameter values. Chains prior to the dashed black line were from pilot iterations used to generate the multivariate normal proposal distribution used for iterations 10,000 and onward.

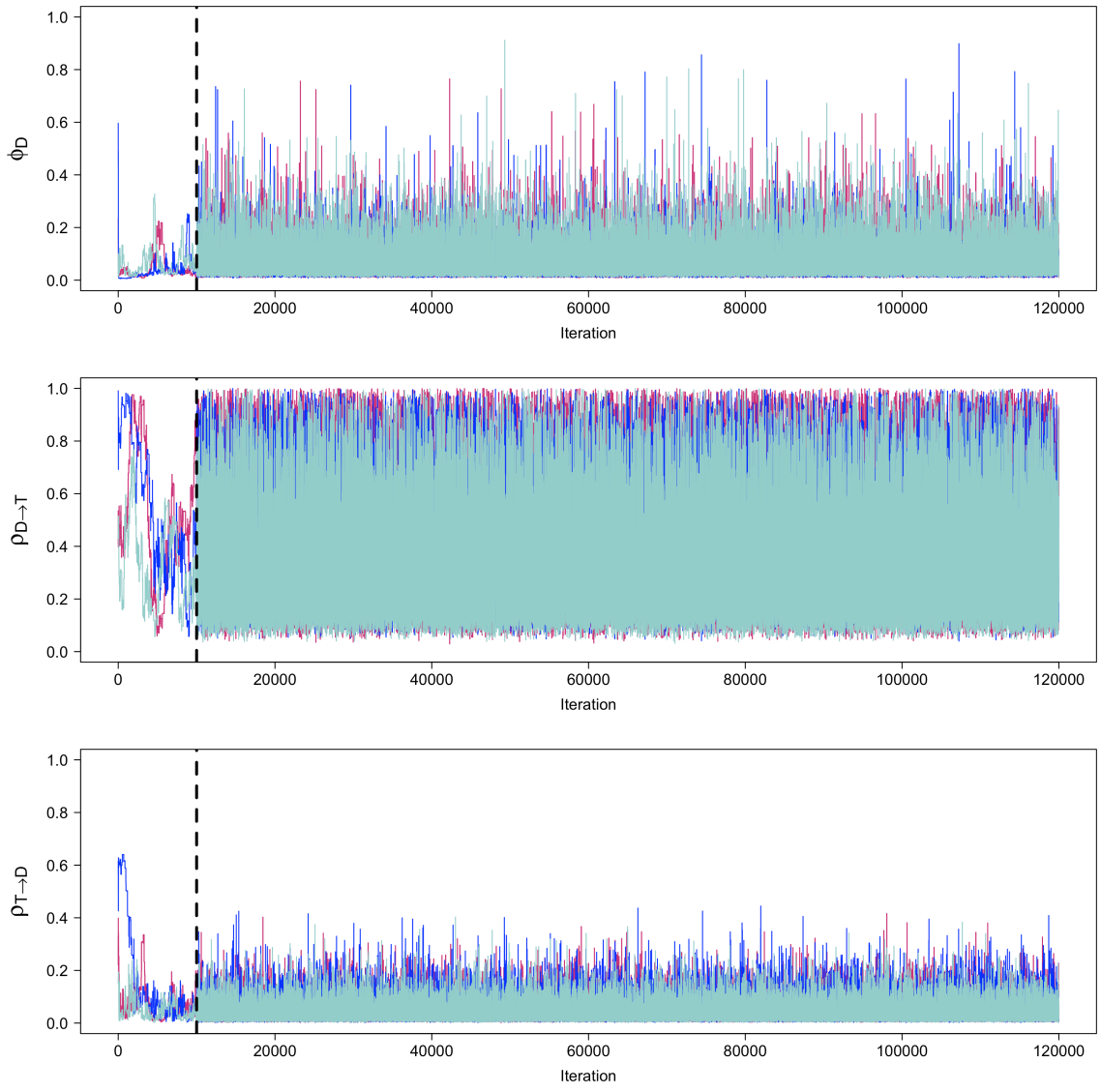

Figure H.3: Scenario 3 relative abundance chains.
Different colors represent chains seeded by different parameter values. Chains prior to the dashed black line were from pilot iterations used to generate the multivariate normal proposal distribution used for iterations 10,000 and onward.

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